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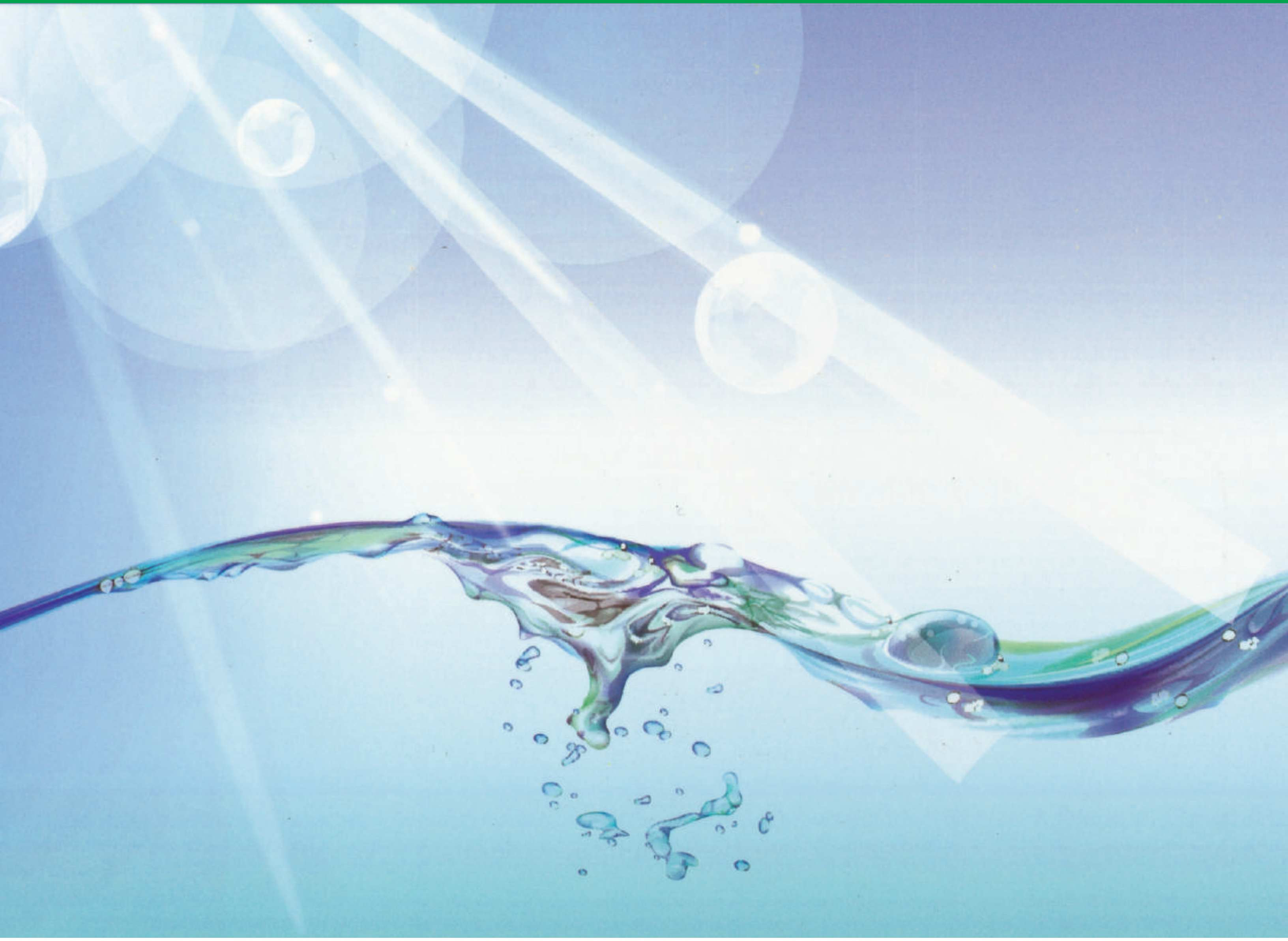

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Genome Wide Association Study in Pulses for Salt Tolerance: Status and Perspective

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Abstract

Genome wide association study (GWAS) is a powerful, reliable, leading and effective approach for unraveling the genetic makeup of complicated phenotypic features back to their underlying genetics. Morphological, physiological, biochemical and quality traits of pulses are severely affected by biotic and abiotic stresses significantly affecting their growth, yield and productivity. Soli salinity is most challenging factor among all abiotic stresses that significantly reduces the yield of pulses. These are a promising source for human diet due to their richness in the content of protein, micronutrients (Fe, Zn etc.), carbohydrates, fibres and antioxidants in addition to their long shelf life, suitability to marginal environments etc. It is crucial to comprehend the genetic foundation of underlying features to boost the production of pulses for world food and nutritional security. Implementing GWAS in pulses can lead to the effective discovery of countless genomic areas linked to salinity stress. It have been applied in pulses to dissect the traits related to yield, such as seed size and pod number, traits associated with resistance to biotic and abiotic stresses, like disease resistance, drought and salt tolerance as well as for micronutrients like Fe, Zn etc. This review presents the overview of the application of GWAS for the pulse's improvement programme, its strengths, weakness and applications that can facilitate the dissection of the gene networks underlying complex traits like salinity and alkalinity and improve its efficiency in a molecular breeding programme.

Key words: Pulses, Salt tolerance, Association mapping, Linkage disequilibrium, Marker trait association, Single nucleotide polymorphism

Introduction

Pulses are the crucial crops among food crops- as they are key sources of proteins (20–25%), minerals [iron (Fe), magnesium (Mg), potassium (K), phosphorus (P), and zinc (Zn)], omega-3 fatty acids and vitamins (Iriti *et al.*, 2017; Langyan *et al.*, 2022). Over the past few decades, industrialization and overpopulation are the key factors responsible for reduced productivity in the agricultural sector (Anderson *et al.*, 2010). Various factors are responsible for low productivity directly impacting food security worldwide, including biotic and abiotic factors (Bennett *et al.,* 2012; Rahman *et al.*, 2017; Farooq *et al.*, 2022). Biotic factors include fungi, bacteria, viruses, nematodes etc. while abiotic factors include disorders caused by drought, salt, cold, and mineral toxicity or deficiency (Kalve *et al.*, 2022). Food and nutritional security are the great challenges ahead in agriculture. So, being rich in nutritions, pulses have a better adaptation for environment to promote dietary and production diversity that significantly reduce malnutrition and hidden hunger (Ritchie *et al.*, 2018). Among pulses, major contributing crops are Chickpea (*Cicer arietinum,* 2n = 2x = 16, ~ 740 Mbp), Common bean (*Phaseolus vulgaris*, 2n = 22, ~ 530 mbp Genome size), Lentil (*Lens culinaris*, 2n=14, ~ 4 Gb), Green gram (*Vigna radiata*, 2n = 22, ~ 494 to 579 Mbp), Red gram (*Cajanus cajan,* 2n = 22, ~ 852 Mbp), Faba bean (*Vicia faba*, 2n = 2x = 12, ~ 13 Gb), and cowpea (*Vigna unguiculata, 2n = 22, ~ 613 Mbp).* Chickpea and lentil are nutritionally rich *rabi* season crops with remarkable sources of protein (20–30%), low digestible carbohydrates

(20%), fat (1%), iron (Fe), zinc (Zn), and a range of vitamins. Pulses are severely affected by biotic and abiotic factors. So, the lower yield level of pulses provides an excellent opportunity to enhance its productivity by implementing new innovative technologies and subsequent popularization and adoption.

Among all abiotic stresses, salinity and alkalinity are the most challenging factors that affect the plants as well as soil health i.e. alarmingly increasing with each passing year. In world, 932.2 million ha area is affected with salinity and sodicity stresses (Metternicht and Zinck, 2003), out of which salinity and sodicity stresses covers an area of nearly 6.73 million hectares in India (Singh *et al.,* 2014). An excess amount of salts occurs as an abiotic environmental factor in many places such as salt deserts in the arid and semi-arid areas, coastal salt marshes and inland saline lakes and areas near canals (Kumar, 2013). Salt stress significantly affects the seed yield mainly because crops exhibit slower growth rates, reduced tillering and, over months, reproductive development is affected (Munns and Tester, 2008). The critical objective for salt tolerance research is to improve the ability of plants to sustain under saline soil. Resistance or tolerance mechanism is complex trait so integrating morphological, physiological, and biochemical alternations and quantitative at the genetics level becomes necessary. The most direct approach to improve pulses productivity for stress is to identifying and increasing the presence of novel genes and alleles associated with it in commercially relevant germplasm. Several studies in pulses in reconciliation to different types of stress have been undertaken. To breed varieties with stable grain nutrient content and yield under stress conditions, a deep insight into the genetic basis of yield, its attributing traits, nutrient content and associated tolerance mechanism is necessary. During last couple of years, huge focus has been on implementing NGS (next generation sequencing) techniques for genetic upgradation that include DNA markers, advanced sequencing technologies and bioinformatics tools. Integration of classical breeding approaches with new generation genomics and phonemics tools with generation acceleration protocols can hasten the progress in the pulse crop improvement programme. Legumes like Chickpea (estimated coverage 72%, total gene 228269 with genome assembly length 532 Mb); Common bean (521.1 Mb genome assembly length, estimated coverage 81.8% having total genes 31638) and Pigeon pea (605.78 Mb genome assembly length with estimated coverage 72.70% and total genes 48680) etc. are explored (Varshney *et al.*, 2013). There are two tactics for marker-trait association: (1). Linkage/QTL Mapping (2). Association/LD Mapping (Xu *et al.*, 2017). Association mapping is categorized into two groups (a). Candidate gene-based association mapping (b). Whole genome or GWAS mapping (Ibrahim *et al.*, 2020). GWAS based on LD (linkage disequilibrium) is an effective approach for mapping in diversity panel and has been applied in several types of crop species. It can detect causal genetic loci of stress related traits by screening large, diverse set of accessions. A narrow genetic base and limited information of the association studies for stress have created a significant bottleneck for pulses improvement. In this context, this review presents the necessitate for conducting a GWAS study in pulses, statistical models, and approaches followed by molecular breeding.

In the present review, breeding methodologies to combat salt stress, the detailed information about GWAS, statistical approaches, linkage disequilibrium, population structure, genomic control, different types of GWAS models and the application of it in the haplotype breeding and marker assisted selection for salinity tolerance has been given. We also tried to cover the all over studies carried for different traits in pulses. GWAS have been studied in other crops but limited information is available for salt tolerance in pulses. To breed varieties with yield under salt stress conditions, a deep insight into the genetic basis of yield, its attributing traits and associated salt tolerance mechanism is necessary and GWAS is the best approach to assist it. Various DNA markers and trait connections have been discovered using traditional linkage mapping approaches but GWAS is the best alternative to mitigate the limitations of others such as resolution, efforts cost and time.

Breeding Methodologies in Pulses for Salinity Tolerance

Salinity tolerance in pulses can be achieved through various breeding approaches, including traditional breeding methods and modern biotechnological techniques. For example, screening and selection, marker-assisted breeding, genomic selection, hybridization, introgression of salt tolerance genes, mutagenesis, transgenic approaches, multi-trait breeding, and participatory plant breeding. Breeding for salinity tolerance in pulses is a complex process that requires a combination of these approaches and continuous evaluation in field conditions. It is crucial to develop and deploy salt-tolerant pulse varieties that can thrive in saline soils and contribute to food security in affected regions. Molecular techniques offer several advantages over traditional breeding methods in crop improvement. While both approaches have their strengths and limitations, molecular techniques have the potential to accelerate and enhance the breeding process in several ways. GWAS provided an advantage over genetic and linkage mapping regarding resolution, efforts etc. It differs from QTL mapping, as QTL mapping relies on principle of linkage analysis i.e., co-segregation of marker and trait due to tight linkage. QTL mapping requires the development of a specific mapping population as per the objective so it requires time effort and cost, only two alleles are considered at a time, low resolution (>10 cM) and population-specific (Liu *et al.*, 2016; Alqudah *et al.*, 2020). GWAS and QTL Mapping are considered complementary as when conducted together, both take the edge off each other's limitations.

Improvement of Pulses for Salinity Tolerance Using GWAS

GWAS was introduced nearly one decade ago in human genetics by Klein *et al.* (2005) with nearly ~1600 published GWAS studies. GWAS is a statistical approach used in genetics to identify genetic variants (usually single nucleotide polymorphisms or SNPs) that are associated with specific traits or diseases across a large and diverse population. By comparing the genetic variations of individuals with their phenotypic traits, GWAS helps to uncover links between genes and complex traits, offering insights into the genetic basis of various conditions and traits. GWAS typically involve an ample number of genetic markers spread across the genome. These markers are genotyped in a diverse panel of pulse crop accessions or breeding lines. Statistical methods are then used to assess the association between genetic markers and trait variations (Brachi *et al.*, 2010; Zhao *et al.*, 2007). The methodology followed to conduct GWAS study is presented in Fig. 1.

Certain pulse crops continue to be underutilized because of factors such as limited information of genomic resources. LegumeInfo is a comprehensive resource that includes genomic data for various legume crops, including many pulse crops and model legume (Berendzen *et al.*, 2021). Some pulse crops have specific genomic databases dedicated to their research e.g., Chickpea Genomics Database (CGD) for chickpeas and Lentil Genome Database (LGD) for lentils. KnowPulse offers comprehensive data on genetic markers, sequence variations, phenotypic characteristics, and germplasm collections for a range of pulse crops, including chickpea, common bean, field pea, faba bean, and lentil (Sanderson *et al.*, 2019). NCBI and GenBank host a wealth of genomic data, including sequences and annotations for various pulse crops. Researchers can access these resources to retrieve genetic information for GWAS. SSR (Simple Sequence Repeats) markers are valuable source for assessing genetic diversity and population structure (Fayaz *et al.*, 2022). SNP markers are commonly used in GWAS studies due to their abundance in the genome (Gunjaca *et al.*, 2021). Some pulse crops have genotyping arrays specifically designed for high-throughput SNP genotyping, facilitating GWAS. These arrays often include thousands of markers. Genetic resources (Databases) of pulses and their sources are presented in Table 1.

Linkage disequilibrium (LD)

LD also termed as GPD (Gametic phase disequilibrium) is different from linkage, where,

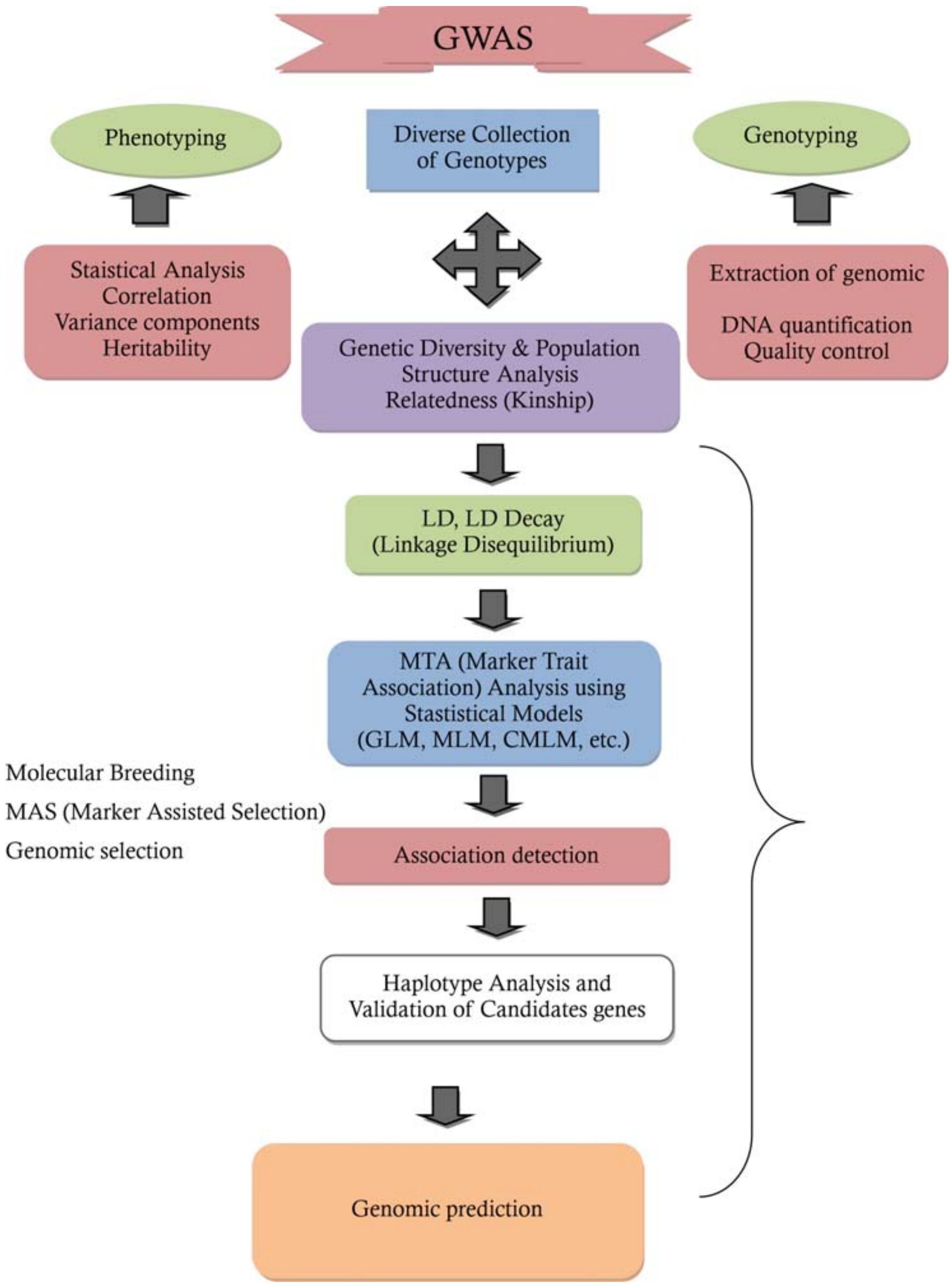

Fig. 1 Workflow of Genome Wide Association Study (GWAS) followed by application in Molecular Breeding

alleles of two genes are inherited together because they are located close to each other whereas LD is a Non-random association between alleles of two loci in a population irrespective of their physical location in the genome. Non-random association occurs due to the presence of random drift, mutation, population bottleneck, small size population, nonrandom mating and natural selection. Although LD would be generated by linkage between loci, considerable LD can be detected between even unlinked genes due to epistatic selection. Epistatic selection occurs when the fitness or phenotypic effect of one gene depends on the alleles at other unlinked loci. This can lead to non-random associations between alleles at different loci, even if they are on different chromosomes. These non-random associations can create or maintain LD between unlinked genes. As a result, the allelic combination of the aforementioned loci seen in the population deviates greatly from their predicted frequencies based on independent assortment.

Table 1. List of databases and their tools for pulses

S.N.	Database	Crop species	Tools	References
1.	Legume Federation	Various species of legumes	Annotation, Sequence Search, Genes and Gene Families, Synteny, Germplasm, Genetic Markers, Genetic Maps and Genome Viewers, QTLs, Expression, Genetic Variant Data, GWAS viewer	*https://www.legumefederation.org/en/*
2.	Pulse Crop Database: PulseDb (PCD)	Various species of pulses	BLAST, Map viewer, Synteny viewer, PathwayCyc, JBrowse BIMS etc.	*https://www.pulsedb.org/*
3.	LegumeIP	Pulses and model legumes (e.g., *Lotus japonicus*)	Gene Function and Genome Evolution in Legumes	*https://plantgrn.noble.org/LegumeIP/gdp/*
4.	Phytozome	Various species of cereals, pulses, horticultural crops	BLAST, PhytoMine, Synteny etc.	*https://phytozome.jgi.doe.gov*
5.	PeanutBase	*Arachis hypogaea*	BLAST, ArachisMine, Legumebot, Gene search, Funnotate, Genome context viewer, Gene expression resources etc.	*https://peanutbase.org/*
6.	Know Pulse	Various species of pulses	Diversity Data for pulse crop improvement	*https://knowpulse.usask.ca*
7.	Legume Information System (LIS)	Pulses and model legumes (e.g., *Lotus japonicus*)	LIS Intermines, LIS Datastore, Gene search, Funnotate, Genome context viewer, Genetic Map viewer, Gene family Search, GCViT: SNP Comparison Tool etc.	*https://legumeinfo.org*
8.	Vigna Genome Server	Species of the *Vigna* genus such as *Vigna unguiculata*	BLAST, BLAT, Genome Browser etc.	*https://viggs.dna.affrc.go.jp*
9.	SoyBase	*Glycine max*	BLAST, Pangenome, Ontology, Metabolic pathways, Pedigree database, SCNBase, GRIN Accessions Map etc.	*https://www.soybase.org*
10.	SoyKb	*Glycine max*	BLAST, Phylogeny, Affymetrix, Pathway Viewer, Protein bioviewer, MADis tool, Mutant Finder etc.	*http://soykb.org/*
11.	Chikpea Database (CTDB)	*Cicer arietinum*	Transcriptome sequence, Functional annotation, Conserved domain(s), Transcription factor families, Molecular markers (microsatellites and single nucleotide polymorphisms), Comprehensive gene expression and comparative genomics	*http://nipgr.res.in/ctdb.html*

pAB ≠ pA.pB, pAB.pab ≠ pAb.paB (if population is in LD)

D= pab.pab-pAb.paB

LD is estimated as Lewontin's ('D') and coefficient of correlation (r^2) (Hindu *et al.*, 2018). In Arabidopsis, LD generally decays 50% within 5 Kb whether two SNPs are strong or weak LD can be determined using r^2 estimate. The Value of r^2 greater than 0.2, presence of two SNPs on same QTL as well as their co-inheritance revealed Low LD enables high resolution mapping by depicting only those loci which are tightly linked to the gene of interest having significant association with the trait of interest (Alqudah *et al.*, 2020). The specific LD decay value for pulses can vary among different populations and varieties of the plant. Therefore, we would need access to genetic data from your specific population of interest and conduct a LD analysis to determine the LD decay value for a particular study. Some of the following studies have been conducted to determine LD decay that ranged from 80-100 kb in cowpea (Sodedji *et al.*, 2021), 500-600 kb (Saxena *et al.*, 2014) and 150-200 kb (Upadhyaya *et al.*, 2016) in chickpea, ~ 4 Mb in common bean

(Ugwuanyi *et. al.*, 2022), 60-100 kb (Noble *et al.*, 2018), 384 kb (Seo *et al.*, 2023), 57.6 kb (Sinha *et al.*, 2023) and 105 kb (Sokolkova *et al.*, 2020) in green gram.

Statistical approaches for GWAS

Statistical methods, software and models play important roles in association study. TASSEL, EMMAX, GenAMap, GenABEL, FaST-LMM, GAPIT, STRUCTURE, SPAGeDI, EINGENSTRAT, MTDFREML, R and other commercial available packages like ASREML, Genstat, JMP Genomics and SAS are diverse type of software used in GWAS study. Kinship plays a crucial role in controlling for genetic relatedness among individuals in the study of population. By assessing the degree of kinship, researchers can reduce the risk of false-positive associations, as closely related individuals may share genetic variants that affect the trait of interest. Methods like Principal Component Analysis (PCA), sample correlation based genomic relationship matrix (sc-GRM) etc. are generally used to estimate kinship (Jiang *et al.*, 2022). Properly addressing kinship enhances the accuracy and reliability of GWAS results.

GWAS in Pulses for Different Traits

GWAS have become powerful tools for identifying genetic variants associated with various traits viz. yield, disease resistance, drought/heat/frost/mineral toxicity/salt tolerance, nutritional traits, seed size and shape, flowering time, root traits, pod traits etc. GWAS for different traits in pulse crops involve large-scale genotyping of a diverse population of individuals, followed by statistical analysis to identify associations between genetic variants and specific traits of interest. GWAS findings can provide valuable deep understanding of the genetic architecture underlying various traits, helping plant breeders to develop improved pulse crop varieties by following MAS or other genome editing techniques. The findings of GWAS in different pulses are presented in Table 2. And number of studies during last one decade presented in Fig. 2.

Chickpea

GWAS have remarkable study in chickpea to identify genes controlling traits such as branch number (Basu *et al.*, 2019), Fe and Zn content (Srungarapu *et al.*, 2022), physiological P-use efficiency, shoot dry weight and shoot P content (Thudi *et al.*, 2021), Aschochyta blight resistance (Li *et al.*, 2017; Raman *et al.*, 2022), salt stress related long non-coding RNAs (lncRNAs) (Kumar *et al.*, 2021), drought and heat (Kalve *et al.*, 2022), auxin related genes associated with yield and yield-related traits under drought-prone environments (Li *et al.*, 2018), drought and heat stresses (Thudi *et al.*, 2014), *P. thornei* resistance (Channale *et al.*, 2023) and salinity tolerance etc (Ahmed *et al.*, 2021).

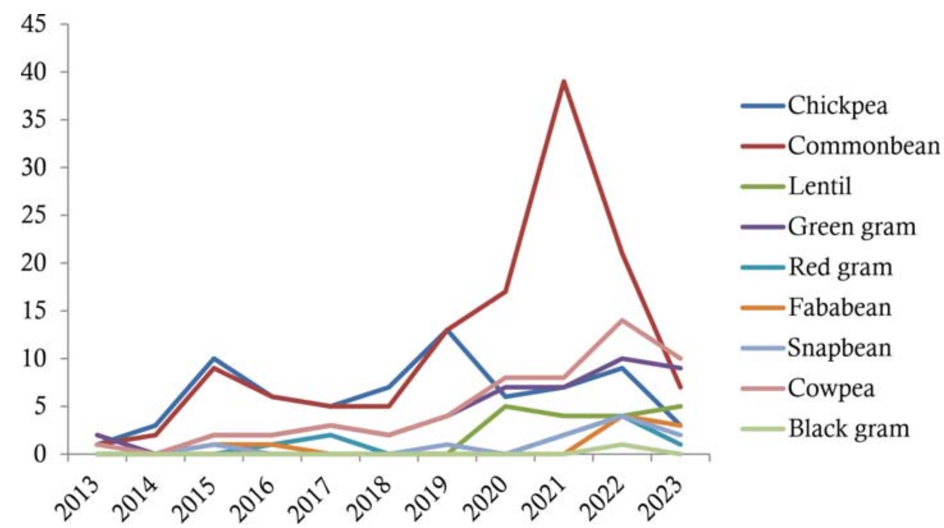

Fig. 2 Genome-wide association study (GWAS) in different pulses worldwide over the last ten years (2013-2023)
Source: PubMed (keywords "GWAS in Pulse crop wise" were used to search the number of publications in PubMed)

Table 2. GWAS studies for different traits in pulses

S.N.	Crop	Traits	Model used	No. of MTAs Identified	Genomic Region	References
1.	Chickpea	Protein, Fe and Zn content and Agronomical Traits	FarmCPU	20 and 46	Chr: 1, 4, 6, and 7	Srungarapu *et al.*, 2022
		Cu	MLM	35	Chr: 1,2,6	Fayaz *et al.,* 2022
		β-Carotene, Crude protein, Calcium and folate	MLM, MLMM	62	Ca1, Ca3, Ca4, and Ca6	Roorkiwal *et al.*, 2022
		Resistance to *Ascochyta rabiei*	MLM, CMLM and Super-MLM	30	Ca1, Ca2, Ca6 and Ca7	Farahani *et al.*, 2022
		Salinity tolerance phenological,	MLM	-	Ca2, Ca4	Ahmed *et al.*, 2021
		physiological and yield traits under high temperature stress	MLM	-	Ca1, Ca2, Ca4, Ca6	Jha *et al.*, 2021
		Grain Fe and protein under drought and non-stress	MLM	181	Chr: 1, 4	Samineni *et al.*, 2021
		Drought tolerance	MLM	38	Ca2,3,4,5,6	Li *et al.*, 2018
		Fe, Zn	MLM	8	Chr: 1, 4, 7	Diapari *et al.*, 2014
2.	Common bean	Pod morphological and colour characters	MLM	62	Telomeric region of chr: Pv01, Pv02, Pv04, Pv08, Pv09 and Pv10	García-Fernández *et al.*, 2021
		N, P, K, Ca, Mg, Fe, Zn, and Mn	MLMM	22	Pv03, Pv05, Pv07, Pv08, Pv09 and Pv10	Gunjaèa *et al.*, 2021
		Bean fly resistance	CMLM	83	*Pv01* and *Pv10*	Nkhata *et al.*, 2021
		Bean fly resistance	GLS	6	Pv01 and Pv09	Ojwang *et al.*, 2021
		Resistance to *Sclerotinia sclerotiorum*	SL-GWASML-GWAS:mrMLM, FASTmrMLM FASTmrEMM	22	Chr: Pv01, Pv02, Pv03, Pv04, Pv08, and Pv09	Campa *et al.*, 2020
		Flowering time variation	MLM	8	Chr: Pv01, Pv04, Pv06, Pv08	Raggi *et al.*, 2019
		Production traits under abiotic stress	MLM	33	Chr: 1,2,3,4, 8,11	Oladzad *et al.*, 2019
		Anthracnose and angular leaf spot resistance	MLM	2 and 1	Pv-02	Fritsche-Neto *et al.*, 2019
		Anthracnose and angular leaf spot	MLM	17 and 11	Chr: Pv03, Pv04, Pv08	Perseguini *et al.*, 2016
3.	Lentil	Seed Fe and Zn content	FarmCPU	23 and 14	Chr: 1,2,4,5,6	Singh *et al.,* 2023
		Phenology related traits	MLM, MLMM, FARM CPU	-	Chr: 2, 5,6	Neupane *et al.*, 2023
		Stemphylium blight resistance	FarmCPU	12	Chr: 1, 2, 3, 5, and 7	Adobor *et al.*, 2022
		Salt tolerance	MLM	26	Chr: 2 and 4	Dissanayake *et al.*, 2021
		Days to flowering, days to maturity, seed per pod, seed weight	GLM	13	Chr: 2, 3, 5, 6, 7	Rajendran *et al.*, 2021
		Seed Fe and Zn content	GLM	3 and 4		Singh *et al.*, 2017

Contd...

S.N.	Crop	Traits	Model used	No. of MTAs Identified	Genomic Region	References
4.	Snap bean	White mold resistance	FarmCPU	34	Chr Pv07	Arkwazee *et al.*, 2022
		Total phenolic content	FarmCPU	11	Chr: Pv07	Myres *et al.*, 2019
5.	Cowpea	Seed protein content	SMR, GLM, MLM, FarmCPU	7	Chr: 8	Chen *et al.*, 2023
		Alkali stress tolerance	-	32	-	Xu *et al.*, 2023
		Resistance to aphids	GLM, MLM, FarmCPU	5	Chr: Vu02, Vu 08, Vu 10	Ongom *et al.*, 2022
		Salt tolerance	FarmCPU	79	Chr: 3, 7	Ravelombola *et al.*, 2022
		Salt tolerance	GLM, MLM, FarmCPU	9	Chr: 1, 3, 5	Liu *et al.*, 2022
		Drought tolerance index	FarmCPU	77	Chr: 8	Ravelombola *et al.*, 2021
		Flowering time	GLM, MLM	7	Chr: Vu 01, 2, 4, 7, 8, 9, 10	Paudel *et al.*, 2021
		Seed size	MLM	17	Chr: 3	Lo *et al.*, 2019
6.	Faba bean	Drought tolerance	MLMM	29	Chr: 1, 2	Gutierrez *et al.*, 2023
		Root traits associated with frost tolerance	GLM	9	Chr: 1, 3, 5, 6	Sallam *et al.*, 2022
		Herbicide tolerance	ST and MT-GWAS	20	-	Maalouf *et al.*, 2022
		Herbicide tolerance	ST and MT-GWAS	24	-	Abou-Khater *et al.*, 2022
		Frost tolerance	GLM, MLM	54	Chr: 2, 3, 4, 5, 7	Sallam *et al.*, 2016
7.	Green gram	Grain micronutrients and anti-nutritional traits	GLM, Blink	20	Chr: 1,2,3,4,5, 6,7,8,9,10	Sinha *et al.*, 2023
		Drought tolerance	MLM	146	-	Chang *et al.*, 2023
		Flowering time	-	7	Chr: 2	Seo *et al.*, 2023
		Agronomical traits	GLM, MLM	13	Chr: 7	Kumari *et al.*, 2022
		Agronomical traits	-	110	Chr: 3, 4, 5	Han *et al.*, 2022
		Yellow Mosaic Virus	GLM, MLM	15	Chr: LG2, 4, 9	Singh *et al.*, 2020
		Salinity tolerance	MLM, FarmCPU	5	Chr: 7, 9	Breria *et al.*, 2020
		Phosphorous use efficiency	GLM, MLM	136	Chr; 4, 7, 8	Reddy *et al.*, 2020
8.	Black gram	Yield and yield related traits	Farm CPU, MLM	49	Chr 4, 6, 8, 11	Singh *et al.*, 2022
9.	Red gram	Agronomical trait	-	92	Chr: 6	Zhao *et al.*, 2022
		Flowering traits	GLM, MLM, MLMM, CMLM, EMLM, Farm CPU and SUPER	22	Chr: 2, 6	Kumar *et al.*, 2022
		Agronomical trait	-	14 to 133	Chr: 2, 5	Kinhoégbè *et al.*, 2022
		Resistance to Fusarium wilt	MLM	6		Patil *et al.*, 2017

Common bean

GWAS has been used to identify genes controlling traits in common bean such as biotic stress (Shi *et al.*, 2011; Perseguini *et al.*, 2016; Zuiderveen *et al.*, 2016; Tock *et al.*, 2017, Fritsche-Neto *et al.*, 2019), drought stress (Galeano *et al.*, 2012; Hoyos-Villegas *et al.,* 2017), rust resistance in the common bean (*Phaseolus vulgaris* L.) (Wu *et al.,* 2022), resistance to beet curly top virus (Soler-Garzón *et al.*, 2023), morphological traits (Nemli *et al.*, 2014; Kamfwa *et al.*, 2015a; Moghaddam *et al.*, 2016; Ates *et al.*, 2018; Nascimento *et al.*, 2018; Resende *et al.*, 2018), N fixation (Kamfwa *et al.*, 2015b), cooking time (Cichy *et al.*, 2015), flooding tolerance (Soltani *et al.*, 2017; Soltani *et al.*, 2018), pod shattering (Rau *et al.*, 2019) and Fe, Zn (Mahajan *et al.*, 2017; Katuuramu *et al.*, 2018; Myers *et al.*, 2019; Diaz *et al.*, 2020; Erdogmus *et al.*, 2020).

Lentil

GWAS has been used to identify genes controlling traits in lentil such as seed Fe and Zn content (Singh *et al.*, 2023), anthracnose race 1 resistance (Gela *et al.*, 2021), *Aphanomyces* root rot resistance (Ma *et al.*, 2020), prebiotic carbohydrates and crop stress tolerance (Johnson *et al.*, 2021), root rot caused by *Fusarium avenaceum* (Heineck *et al.*, 2021), ascochyta blight (AB) resistance (Henares *et al.*, 2023), phenology-related traits (Neupane *et al.*, 2023).

Snapbean

GWAS has been used to identify genes controlling traits in snap bean such as white mold resistance (Arkwazee *et al.*, 2022), pod stringlessness (Liu *et al.*, 2022), total phenolic content (Myers *et al.*, 2019), pod size (Li *et al.*, 2023), morpho-agronomic and seed quality traits (Ugwuanyi *et al.*, 2022), root rot caused by *Fusarium solani* (Mart.) *f. sp. phaseoli* (Burkholder) (Huster *et al.*, 2021).

Cowpea

GWAS has been used to identify genes in cowpea controlling traits such as seed protein content (Chen *et al.*, 2023), resistance to aphids (*Aphis craccivora*) (Ongom *et al.*, 2022), salt tolerance (Ravelombola *et al.*, 2022), flowering time (Paudel *et al.*, 2021), seed size (Lo *et al.*, 2019), drought tolerance index (Ravelombola *et al.*, 2021), salt tolerance (Liu *et al.*, 2022), alkali stress tolerance (Xu *et al.*, 2023).

Faba bean

GWAS has been used to identify genes in faba bean controlling traits such as heat stress tolerance (Maalouf *et al.*, 2022), frost tolerance (Sallam *et al.*, 2016), drought tolerance and associated traits (Gutiérrez *et al.*, 2023), herbicide tolerance (Abou-Khater *et al.*, 2022), root traits associated with frost tolerance (Sallam *et al.*, 2022).

Red gram

GWAS has been used to identify genes in red gram controlling traits such as days to flowering (Kumar *et. al.*, 2022), agronomical traits (Zhao *et al.*, 2022; Kinhoegbe *et al.*, 2022), epigenetic and transcriptional changes associated with heterosis (Sinha *et al.*, 2020) and resistance to fusarium wilt (Patil *et al.*, 2017).

Black gram

GWAS has been used to identify genes in black gram controlling yield and yield attributing traits such as days to 50 % flowering, days to 90% pod maturity, plant height, branches per plant, nodes per plant, internodal length, clusters per plant, pods per plant, pod length, seeds per pod, yield per plant, seed weight and harvest index and identified the interested loci on the chromosome number chr 4, chr6, chr 8 and chr 11 (Singh *et al.,* 2022).

Green gram

GWAS has been used to identify genes in green gram controlling traits such as phosphorous use efficiency (Reddy *et al.*, 2020), grain micronutrients and anti-nutritional traits (Sinha *et al.*, 2023), drought tolerance (Chang *et al.*, 2023), salinity tolerance (Breria *et al.*, 2020), flowering time (Seo *et al.*, 2023), yellow mosaic virus (Singh *et al.*, 2020), agronomical traits (Han *et al.*, 2022; Kumari *et al.*, 2022).

Data Quality Control

Data quality control is crucial for GWAS analysis, ensuring data validity through statistical procedures and filtering. These procedures are performed on individuals and SNPs, with those not meeting criteria deleted. PLINK is a freely available tool for large-scale GWAS data analysis with management and analysis (Purcell *et al.*, 2007). Missing call rate, individual independence, Minor Allele Frequency (MAF), Hardy Weinberg Equilibrium (HWE) factors are considered for data quality control. Individuals that have high missing call rates (fraction of SNPs whose genotypes are not called for a specific individual) indicate poor DNA quality. Missing call rates >1% - 5% are removed. Alternative solutions for dealing with missing SNPs include data imputation, replacing missing markers with their expectations, testing untyped SNPs, and meta-analysis (Howie *et al.*, 2012).

Individual independence is taken into consideration during association study as hidden relationships may lead to false associations. PLINK is used to calculate PIHAT (for the purpose of finding duplicates and likely relatives) based on pair-wise identify-by-state (IBS). High PIHAT individuals (e.g., PIHAT > 0.25) are eliminated. Rare variants with MAF <1% are error-prone, have low statistical influence, may result in unauthentic associations so excluded from analysis (Wang *et al.*, 2005). GWAS is based on linkage disequilibrium, it becomes necessary to verify whether it deviates from HWE for true associations. The chi-square test is conducted for each one SNP and p-value is calculated (Wigginton *et al.*, 2005). The threshold of $-\log_{10}$ (p-value) can be fixed at a confidence value of which $-\log_{10} > 3$ is the most common and reliable value. Mendelian error, heterozygosity, population outliers should be verified for true association study. These quality control threshold criteria differ according to case studies. It relies on the population size, Genotyping markers, platform etc.

Population Structure

Population structure is also termed population stratification/genetic structure, which is the occurrence of orderly ancestral differences in allelic frequencies between subpopulations. In addition to it, family structure, Population admixture and cryptic relatedness are other terms used to describe population structure. To identify and account for the potential population structure, several methods have been put forth (Setakis *et al.*, 2006; Cardon and Palmer, 2005; Vilhjalmsson and Nordborg, 2015). Subpopulations in population are detected by STRUCTURE software (Pritchard *et al.*, 2000). Genomic control approaches and principal component analysis (PCA) generally used to analyze the population structure. Genomic control is a statistical method that allows us to evaluate the degree to which population structure affects the association study. It corrects the population structure by considering the chi-square test (X^2) and constant inflation factor (λ) i.e., using X^2/λ in place of chi-square. Since a value higher than one suggests population structure, should be equal to one in a uniform population.

$$\lambda = \text{Median}\,(X^2_1, X^2_2, X^2_3 \ldots\ldots X^2_L)/0.456$$

Here, L is selected less than or equal to the number of SNPs, m and 0.456 is the expected median of the chi-squared distribution with d.f. = 1.

PCA is considered the gold standard method for population structure analysis and genetic relatedness that limits the confounding effect in GWAS. By removing the top several independent axes of variation, it captures the genetic background of concealed ancestral ancestry. Singular value decomposition (SVD) on the genotype Matrix G is used to calculate principle components (PCs) (Price *et al.*, 2006; Elhaik E, 2022).

Association Mapping

The methods used in association analysis are single SNP scan by additive genetic model, linear regression, analysis of variance, t-test for simple association analysis, and multi-marker analysis for complicated characteristics. Several comparisons are critical when dealing with enormous amounts of data. Due to the presumption each genetic variant examined is independent of the others, the bonferroni correction technique is regarded as the most cautious way for determining a threshold P-value (Kaler *et al.*, 2019). Q-Q plot, manhattan plot, locus zoom plot and haploview plot are used as visual presentation tools to view and interpret the results of GWAS. The Manhattan plot indicates the p-value of GWAS. This plot is made by graphing the position of SNPs along with chromosome number on X-axis and $-\log_{10}$ value on the vertical axis (Zeng *et al.*, 2015).

Various types of GWAS models are used to associate genotype with phenotype in pulses ranging from simple to complex. Initially, ANOVA, t-test and regression models were used but resulted in false positive results due to exclusion of proper estimation of population structure and genetic relatedness. To overcome, this issue, different types of models are available like GLM, MLM, CMLM, MMLM, SUPER, FARMCPU, Blink etc. The most common approach for conducting GWAS analysis initially involved the use of GLM (General Linera Model). However, it was later recognized that GLM led to numerous false-positive associations due to its failure to account for population relatedness. As a result, GLM was subsequently replaced by more recent mixed models (Segura *et al.*, 2012). Simulation models demonstrated that MLM outperformed other methods in controlling false positives, but it comes with a significant computational burden. CMLM (Compressed Mixed Linera Model) i.e. intermediate of GLM and MLM, a compressed version of the mixed linear model (MLM), accelerates computations by grouping individuals within the random effect model. It achieves faster processing while maintaining equal or improved statistical power when compared to MLM (Zhang *et al.*, 2010). MLMM and SUPER models include the significant QTNs to eliminate the issue of confounding between the tested markers and kinship. To entirely address the confounding problem in the analysis, Farm CPU was developed by Liu *et al.*, 2016, which partitions the model into both fixed and random effect components. The most recent GWAS model, Bayesian Information and LD Iteratively Nested Keyway (BLINK), effectively resolves the issues that were present in Farm CPU (Huang *et al.*, 2018). BLINK employs Bayesian information criteria to substitute the random effect component of Farm CPU with a fixed effect model. Additionally, it leverages LD information to sequentially introduce one marker at a time into the model, effectively addressing and eliminating the confounding issue (Huang *et al.*, 2018).

Application of GWAS Results in Molecular Breeding

Dissection and application of the genetic dissection of traits related to the improvement of salt tolerance become possible through the appliance of Haplotype breeding, Marker-assisted selection (MAS)/markers assisted based backcrossing (MABC), Precision breeding, Genomic Selection (GS) and other genome editing tools (Nordborg *et al.*, 2008; Mandel *et al.*, 2013). GWAS can identify specific genetic variants or haplotypes associated with salt tolerance traits in crops. A haplotype refers to a grouping of alleles from various polymorphic sites, like SNPs, insertions/deletions, or other genetic markers, located on the same chromosome. These alleles tend to be inherited as a unit with minimal likelihood of undergoing simultaneous recombination events (Bhat *et al.*, 2021). Once associated haplotypes are identified through GWAS, they can serve as markers for accelerated breeding such as Marker assisted selection (MAS). Breeders can use these markers to screen large populations quickly and select individuals with the desired haplotypes, expediting the breeding process (Qian *et al.*, 2017). GWAS results can guide the pyramiding of multiple beneficial haplotypes into a single crop variety (Lv *et al.*, 2021). This can lead to the development of high-performing, salt tolerant cultivars with a combination of desirable traits. Missing heritability is the challenge for its application which requires more molecular markers and the large size of entities to identify a broad range of genomic loci. But this problem is less serious in crop improvement due to some genetic variants, which explicitly demonstrate the phenotypic variation (Brachi *et al.*, 2011). GWAS has the efficiency to be utilized directly in crop improvement programs (Jannink *et al.*, 2010). Yet, it is essential to validate the identified associations through further experimentation and functional studies to confirm their roles in salt tolerance.

Strengths and Limitations of GWAS

GWAS is a powerful approach in genetics that have significantly advanced our understanding of the genetic architecture of various traits and malfunctions during the past ten years in humans, wheat, rice, and other crops etc. However, like any scientific method, GWAS also has its benefits and limitations. Despite evident gains in discovering innovative genes and molecular pathways and their applications, GWAS have not been without criticism. Most association signals represent polymorphisms and genes with little direct biological significance. GWAS is affected by the factors like rare variants with large effects and common with small effects, sample volume, genetic heterogeneity, an imperfect genotype as well as confounding due to genetic relatedness (Korte and Farlow, 2013). GWAS faces the "missing heritability" problem (Manolio *et al*., 2009).

Future Perspectives

GWAS in pulses will continue to be a valuable tool for advancing crop improvement, enhancing resilience to environmental challenges, and addressing nutritional needs. GWAS play a vital role in accelerating the development of improved pulse varieties with traits like salt tolerance, higher yields, disease resistance, drought tolerance, and nutritional quality. Advances in genomics and high-throughput sequencing technologies will enable more precise and targeted breeding strategies. GWAS can help to identify specific genes and genetic variations associated with desired traits, allowing breeders to make informed decisions in selecting parent lines and optimizing breeding programs. Study of specific functions of genes and genetic variants identified through GWAS can provide insight into the underlying molecular mechanisms. As, pulse crops are particularly susceptible to salinity and alkalinity, GWAS can facilitate the creation of resilient varieties by pinpointing genes and haplotypes associated with traits like salt tolerance. These findings enable the development of pulses better suited to changing climatic conditions. GWAS can contribute to the development of pulse varieties with improved nutritional profiles, addressing dietary needs and health concerns. This includes increasing protein content, enhancing micronutrient levels, and reducing anti-nutritional factors.

Conclusions

GWAS have emerged as powerful tools for unraveling the genetic makeup of complex traits in different taxonomical groups specially pulses that have a vital role in global food security. GWAS include the HTP (High Throughput Technology) of diverse pulse crop populations using enormous types of genetic markers over the genome. Statistical approaches are applied to identify significant associations between molecular markers and the traits such as yield, nutritional composition, seed size, seed shape, flowering time, root and pod traits under salt stress etc. By detecting the candidate genes after annotation, it can be further applied in molecular breeding techniques like MAS, Genomic selection and other gene editing tools. However, it requires the validation of the recognized genetic loci for their use in future crop improvement programme. In conclusion, GWAS approach provides a radical way of looking at the complex genetic network underlying trait variation like salt stress in pulse crops. By combing the GWAS with conventional breeding approaches the full potential of pulses can be unlocked that can assist in maintenance of the food security.

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Genetic Improvement of Chickpea through Marker-assisted Selection for Abiotic Stresses

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Abstract

Chickpea, is vital pulse crop under rain-fed conditions and is important source of protein, fibre and healthy fats befitting people globally. But the crop's productivity is hindered by various abiotic stressors like heat, drought, cold, and salinity. To address these challenges, multiple biochemical, physiological, and molecular mechanisms have been explored and multiple genes contributing to enhance abiotic stress tolerance has been identified. Advanced molecular biology tools and techniques like transcriptomics, proteomics, and metabolomics have enabled development of molecular markers which are important for gene/allele introgression. These markers facilitate marker-assisted selection (MAS) for yield components vis-à-vis abiotic stress tolerance and thus make the genetic improvement in chickpea faster. Promising outcomes have emerged from studies involving transgenic chickpea plants and gene editing techniques to develop drought-tolerant chickpea varieties. The present review focusses on use of marker assisted breeding and marker assisted selection to obtain chickpea genotypes tolerant to abiotic stress, especially in the context of climate change. Finally, we propose further research and steps to take in the era of advanced molecular biology techniques.

Key words: Chickpea, Marker Assisted Breeding (MAB), Marker Assisted Selection (MAS), Salinity

Introduction

Globally, over 2.3 billion people, roughly 30% of the world's population, suffer from malnutrition, primarily due to inadequate food intake and nutrient-poor diets (FAOSTAT, 2021; Hawkes, 2017). Chickpea (*Cicer arietinum* L.), rich in protein, fibre, healthy fats, vitamins, and essential nutrients, provide significant health benefits for the growing global population (Kaur and Prasad, 2021). This legume plays a crucial nutritional role in combating various health problems like cardiovascular disease, type 2 diabetes, digestive disorders, and specific cancers, benefiting millions worldwide (Jukanti *et al.,* 2012; Merga *et al.,* 2019). It is a vital cool-season legume crop second only to common beans, having a genome size of 738.09 Mb (Varshney *et al.,* 2013). It primarily thrives in arid and semi-arid regions worldwide, with diverse growth habits like erect, semi-erect, spreading, semi-spreading or prostrate, with branches emanating from the stem (Singh *et al.,* 2008; Sajja *et al.,* 2017). Globally, it ranks as the third most significant pulse crop, cultivated on 15.00 million hectares, yielding 15.89 million tons in 2021 (FAOSTAT, 2023). Over 90% of its production occurs in developing nations, with the Indian subcontinent (India, Pakistan, Myanmar, Bangladesh, and Nepal) contributing nearly 70% of it (Jain *et al.,* 2013).

While chickpea has a yield potential of 6 Mg ha^{-1} under normal conditions, the presence of new challenges like those encountered in new cultivation regions, such as parts of Australia, leads to reduced yields and introduces additional biotic and abiotic challenges (Kaloki and Devasirvatham, 2019; Maphosa *et al.*, 2020). Abiotic stresses, alone or combined, significantly reduce global chickpea yields, especially in arid regions with water scarcity, low rainfall, and soil salinity. Chickpea, typically cultivated in semiarid regions, exhibits high sensitivity to salinity, leading to an annual global yield loss estimated at 8–10%

(Flowers *et al.*, 2010). Drought and extreme temperatures account for substantial proportions of global chickpea yield losses, up to 50% and 20%, respectively (Varshney *et al.*, 2014). In India also, chickpea yield and production exhibit significant yield losses of approximately 70% due to drought, 25% each due to heat and salinity (Mirchandani *et al.*, 2023).

The prevalence of salt-affected soils is a common issue in arid and semiarid regions, attributed to both natural processes and irrigation practices, ultimately affecting substantial land areas (Reynolds *et al.*, 2005). Salinity hampers chickpea growth, affecting germination, flowering, pod formation, and seed filling, with prolonged exposure causing toxic sodium and chloride ion accumulation, leading to leaf senescence and necrosis. (Munns and Tester, 2008; Campbell, 2015). Recent research has emphasized the role of sodium (Na^+) rather than chloride (Cl^-) ions in salt toxicity (Khan *et al.*, 2016; Vadez *et al.*, 2007). To counter ion toxicity, plants employ strategies like sodium exclusion from the transpiration stream, with chickpea genotypes showing significant differences in ion exclusion and tissue tolerance (Maliro *et al.*, 2008). In water-scarce, hot, and saline environments, breeders must develop high-yielding chickpea genotypes using a combination of breeding methods. These new chickpea cultivars must be climate change resilient, genetically diverse, efficient, and widely adaptable to a range of environments to maintain food security in the near and medium-term future (Mba., 2013). Like most pulse crops, research on chickpea improvement has lagged compared to major cereal crops. Agronomic practices such as management of sowing time, in combination with the crop's phenology, can be effectively used to select varieties suitable for different agro ecological zones. The rationale of varying sowing time is to identify varieties that are able to reach the highly sensitive reproductive growth phase when the risks of major abiotic stresses are low. The genes controlling the timing and duration of key growth phases are largely characterized in chickpea and can be effectively deployed in developing new varieties using modern breeding approaches like MAS (marker assisted selection). In this sense, the main focus of this review paper is to provide a comprehensive review of previous efforts to develop abiotic factors (mainly salinity) tolerant chickpea cultivars mainly, through advanced molecular approaches like MAS. It elaborates and provides an overview on conventional and advanced breeding approaches currently being applied to develop salinity tolerant chickpea varieties.

Understanding Abiotic Stress Impact on Chickpea and Harnessing Salinity-Resistant Germplasm

But major abiotic stresses, including extreme temperatures, severe drought, and salinity, pose major constraints to chickpea production, particularly during its reproductive phase, impacting seed production (Lake and Sadras, 2014). These stresses affect different traits, metabolic and physiological processes, and elicit different responses from plants (Table 1). Chickpea's ability to thrive in water-scarce/semi-arid regions is primarily due to its adaptive root distribution, which imparts drought tolerance to it (Benjamin and Nielsen, 2006).

Cold stress in chickpea, particularly when mean daily temperatures drop below 15 °C, leads to flower and pod abortion in specific areas of India and Australia. (Savithri *et al.*, 1980; Srinivasan *et al.*, 1999; Clarke *et al.*, 2004). Winter sowing of chickpea offers increased yield, greater water use efficiency, and improved moisture conditions, but entails the risk of winter killing due to cold stress (Heidarvand *et al.*, 2011). Excessive soil salinity, characterized by surplus soluble salts, induces osmotic stress, specific ion toxicity, and ionic imbalance, ultimately leading to plant death (Munns, 2003; Rout and Shaw, 2001). Thus, abiotic stresses impose the severe effects on plants, and simultaneously, plants possess specific adaptations and regulatory mechanisms to counteract these stresses. At the molecular level, plants initially detect stress through sensing mechanisms, triggering the activation of various pathways through the products of stress-responsive genes to cope with the stress. It is now well known that abiotic stress induces different responses involving stress sensing, signal transduction, and regulation at

Table 1. The main chickpea traits and processes affected by common abiotic stresses

Abiotic stress	Key processes affected	References
Heat	Crop growth rate and duration	Devasirvatham *et al.,* 2015; Jumrani *et al.,* 2014; Kaushal *et al.,* 2013
	Reproductive organs	Kaushal *et al.*, 2013, Devasirvatham *et al.*, 2012; 2013
	Enzymatic activity	Kaushal *et al.,* 2013
Cold	Reproductive organs	Berger *et al.,* 2012; Clarke and Siddique, 2004; Kumar *et al.,* 2011
	Membrane integrity and enzymatic activity	Kumar *et al.,* 2010; Nayyar *et al.,* 2007; Yadav, 2010
	Crop growth rate and duration	Kumar *et al.,* 2010; Kumar *et al.,* 2011; Whish, Castor and Carberry; 2010
	Germination and/or establishment	Jha *et al.,* 2014; Kumar *et al.,* 2011; Yadav, 2010; Croser *et al.,* 2003
	Photosynthesis	Kumar *et al.,* 2011
Drought	Plant growth duration	Ramamoorthy *et al.,* 2016, 2017
	Reproductive growth	Pang *et al.,* 2017; Pushpavalli *et al.,* 2015
	Abscisic acid (ABA) accumulation in the seed or pod	Pang *et al.,* 2017
	Relative water content	Shariatmadari *et al.,* 2017
Salinity	K/Na ratio reduction/Ion toxicity	Lukasz *et al.*, 2019; Davenport *et al.*, 2007; James, Davenport and Munns, 2006; Khan *et al.*, 2016
	Germination and seedling growth	Mann *et al.,* 2019; Anantharaju and Muthiah., 2007
	Photosynthesis	James, Davenport and Munns, 2006, Khan *et al.,* 2015 and 2017

multiple levels. Therefore, plants have evolved mechanisms to adjust their growth to survive and reproduce under stress. To cope up osmotic stress plants send osmotic/oxidative stress signaling (through antioxidant compounds and osmolites) which activates LEA type genes involved in cell protection (stress responsive proteins, chaperones like HSPs and LEA like proteins). At the molecular level to manage ionic stress, in plants the activation of various regulatory pathways is triggered through the products of stress-responsive genes to cope with the stress. The response to oxidative and ionic stress varies among genotypes, leading to sensitive, tolerant, and resistance in different genotypes. The screening and categorization of these genotypes are crucial, as they can serve as valuable repositories of tolerance genes for breeding stress resistance genotypes.

Managing chickpea abiotic stress involves selecting tolerant cultivars from diverse germplasm for hybridization, considering local, exotic, and wild counterparts to identify tolerant genotypes and explore novel survival mechanisms at various levels (physiological, biochemical, molecular) (Amtmann *et al*., 2005). However, addressing challenges related to salinity, heat and drought stress impact on crop performance is difficult due to their complex, polygenic nature (Vinocur and Altman, 2005). Although drought and salinity stress tolerance are the main focus of the paper, chickpea is also affected by various abiotic stresses. Efforts to breed for salt resistance in chickpeas have been somewhat limited (Sharma *et al*., 2004; Vadez *et al.,* 2007), certain sources of salinity tolerance have been identified in India (Kathiria *et al.,* 1997), Pakistan, and Australia (Maliro *et al.,* 2008). Notably, salt-tolerant lines like CSG 88101 and CSG 8927, as identified which exhibited lower levels of Na^+ in their roots compared to sensitive genotypes. In addition, a salinity-tolerant desi variety, Karnal Chana 1 (CSG 8963), suitable for cultivation in soils with electrical conductivity (ECe) between 4 and 6 dS/m, has been released by ICAR-CSSRI, Karnal, India. Particularly, Karnal Chana-1 (CSG 8962), developed by ICAR-CSSRI, Karnal, stands as the world's first and only salt-tolerant chickpea variety. It was released and approved by CVRC in 1998 for cultivation in salt-affected areas of northern India, specifically Zone–II, comprising the states of Haryana, Punjab, Rajasthan, and Uttar Pradesh. This variety matures in 137-150 days and can yield 1.9-2.1 Mg ha^{-1} under normal soil conditions and 1.4-1.6 Mg ha^{-1} in salt-affected soil (ECe 4-6 dS m^{-1} and pH 8.5-9.0). It exhibits resistance against wilt, root rot, drought, and high temperatures, making it suitable for rice-based cropping systems in northern India. Recent efforts involved screening 252 germplasm accessions, including 211 accessions from a mini-core collection, and breeding lines for salinity tolerance at ICRISAT. Among these, the majority of highly tolerant genotypes belonged to the kabuli type, while the majority of highly sensitive accessions were of the desi type (Serraj *et al.,* 2004). Vadez *et al.* (2007) grew two sets of 263 chickpea genotypes simultaneously. One set was harvested at 40 DAS while the other set was harvested at maturity. Data that showed the biomass under salt stress or biomass under salt stress relative to control was not related to either seed yield under salt stress or to the seed yield under salt stress relative to control. This emphasizes the fact that the ûnal assessment of salinity resistance should be based on grain yield. Thus, the salinity-resistant genotypes identiûed based on traits other than seed yield and its component traits (e.g. number of pods per plant) in different studies (Al-Mutata 2003; Karajeh *et al.,* 2003; Serraj *et al.,* 2004; Maliro *et al.,* 2008) need further conûrmation by evaluating these genotypes for seed yield under saline conditions.

Water scarcity disrupts osmotic balance, leading to osmotic stress influenced by drought, salinity, and low temperatures, with low temperatures impacting plant mechanics and chilling stress causing dehydration stress (Farooq *et al*., 2009; Wang *et al*., 2016). Consequently, under chilling, salinity, and drought stress conditions, the vital role of osmotic adjustment mechanisms becomes evident in helping plants maintain higher turgor potential (Zhang *et al*., 1999; Chaves *et al*., 2009). Drought-resistant germplasm, exhibiting osmotic adjustment and increased turgor potential, may offer benefits for coping with chilling and salinity stress. Significant genetic diversity for drought stress tolerance in chickpea has been observed in various parameters related to morphology, physiology, and grain yield, particularly under varying water conditions in field experiments (Krishnamurthy *et al*., 2010; Jha *et al*., 2014; Pang *et al*., 2017). Utilizing field-based screening methods and assessing crop yield performance has led to the identification of numerous chickpea genotypes exhibiting tolerance to both normal and water-stress conditions (Canci and Toker, 2009). Additionally, stress tolerance indices such as the drought susceptibility index and drought tolerance index have unveiled substantial genetic variability in a wide mini-core collection of 211 accessions, spanning various phenological and yield-related traits under water stress (Krishnamurthy *et al*., 2010) (Table 2).

Considering the pivotal role of wild species in conferring drought tolerance, species like *Cicer anatolicum*, *Cicer microphyllum*, and *Cicer songaricum* have been important (Toker *et al*., 2007). Similarly, Kashiwagi *et al*. (2005) identified chickpea landraces across the Mediterranean, West Asian, and Central Asian regions, demonstrating significant genetic diversity in root length density—an attribute that can be leveraged to develop highly water-use-efficient chickpea genotypes under water stress (Condon *et al*., 2004; Zaman-Allah *et al*., 2011a; Zaman-Allah *et al*., 2011b), and substantial genetic variability has been documented in this context (Pang *et al*., 2017). Root architecture traits also play a pivotal role in improving crop performance under drought stress (Wasaya *et al*., 2018; Ye *et al*., 2018) and considerable strides have been made in unravelling the significance of various root traits in conferring drought tolerance in chickpea (Kashiwagi *et al*., 2006; Kashiwagi *et al*., 2015). Attributes such as root biomass, root length, and other root-related parameters, including root length density (RLD), total root dry weight (RDW), and deep root dry weight (deep RDW), have been examined extensively for their contributions to drought tolerance in chickpea (Krishnamurthy *et al*., 2003; Kashiwagi *et al*., 2005; Gaur *et al*., 2008; Kashiwagi *et al*., 2008; Kashiwagi *et al*., 2015; Purushothaman *et al*., 2016; Chen *et al*., 2017). Genotypes like ICC 4958 and ICC 8261, with their extensive RLD, deep root systems, and enhanced root biomass production, have been widely utilized as donor parents to introduce critical drought-adaptive root traits into elite chickpea cultivars, ultimately yielding drought-resilient chickpea cultivars (Gaur *et al*., 2008). Simultaneously, efforts are underway to develop multi-parent advanced generation inter-cross populations (MAGIC) by incorporating genotypes like ICC 4958, JG 130, ICCV 10, JAKI 9218, JG

Table 2. QTLs identified for various abiotic stress related traits in chickpea

Abiotic stress	Mapping approach	Identified QTLs	Type of markers	References
Drought	Biparental	15 QTLs	SSR	Rehman *et al.*, 2011
	Biparental	93 QTLs	SSR	Hamwieh *et al.*, 2013
	Biparental and backcross	'QTL-hotspot'	SSR, AFLP	Varshney *et al.*, 2013
	Biparental	'QTL-hotspot'	SSR	Varshney *et al.*, 2014
	GWAS	312 significant MTAs	DArT, SNP	Thudi *et al.*, 2014
	Biparental	164 main-effect QTLs	SNP, CAPS, dCAPS, SSR	Jaganathan *et al.*, 2015
	Biparental	QTL- hotspot_a (15 genes) QTL- hotspot_b (11 genes)	SNP	Kale *et al.*, 2015
	Biparental	3 candidate genes	SNP	Singh *et al.*, 2016
	Biparental	12 QTLs	SNP	Srivastava *et al.*, 2016
	Biparental	21 QTLs	SNP	Sivasakthi *et al.*, 2018
	GWAS	Several MTAs	SNP	Varshney *et al.*, 2019
Heat	Biparental	4 QTLs	SNP	Paul *et al.*, 2018
	GWAS	Several MTAs	SNP	Varshney *et al.*, 2019
Cold	Biparental	3 QTLs	SNP	Mugabe *et al.*, 2019
Salinity	Biparental	Several QTLs contributing in salinity stress tolerance	SSR	Vadez *et al.*, 2012
	Biparental	-	SSR, SNP	Puspavalli *et al.*, 2015
	Biparental	42QTLs for leaf tissue ion accumulation under salt stress	DArT and SNP	Atieno *et al.*, 2021

130, JG 16, ICCV 97105, and ICCV 00108, each possessing genomic regions/QTLs associated with drought and heat tolerance (Devasirvatham and Tan, 2018). This strategic selection from resultant crosses holds the potential to enhance genetic gains in chickpea under abiotic stresses. Furthermore, Chen *et al.*, (2017) have opened up avenues for improving drought tolerance in chickpea through an extensive assessment of 30 root-related traits and three shoot-related traits in a large core collection.

Though there has been reported the availability of a wide range of genetic variability in chickpea, but modern-day high yielding varieties bred through conventional breeding have uniform genetic base and thus reduction in genetic variability in improved cultivars. Other problems in breeding abiotic stress tolerant genotypes are screening limitations, complex resistance mechanisms, and limited understanding of salinity and environmental interactions in variable conditions. (Flowers and Yeo, 1986). The heterogeneity for salinity in ûelds and the large G X E interaction for salt resistance necessitates screening of test materials over years and locations using a large number of replications in each trial. This makes it difficult to select for salt resistance in segregating populations. Combining conventional and modern methods, including molecular breeding and high-throughput genomics, broadens the genetic base. This integration produces high-yielding varieties resistant to various stresses. Genomic approaches, such as DNA sequencing and genetic profiling, recombinant DNA technology, structural and functional analysis of genome further designing and utilizing molecular markers can play a vital role in creating and detecting new variability for abiotic stress tolerance in plants. In chickpea genomic resources are reported extensively (Singh *et al.*, 2022; Jha *et al.*, 2020; Choudhary *et al.*, 2018) but they are only a few reported of development of new variety using markers. In next section we will discuss in detail about marker assisted breeding and use of markers in chickpea breeding.

Enhancing Chickpea Abiotic Stress Tolerance: Leveraging Marker-Assisted Breeding

Statistical transcriptome sequencing data serves as a valuable resource for developing molecular markers such as microsatellites (SSR) and single nucleotide polymorphisms (SNP). Leveraging advancements in "omics" technologies enables the generation of new datasets for crop plants. This progress facilitates a more integrated association of "omics" data with crop improvement, potentially evolving from genomic-assisted breeding (GAB) to omics-assisted breeding (OAB) in the future (Langridge and Fleury, 2011). This approach can be applied to broaden the genetic bases in chickpea. Application of these omics approaches are Marker-Assisted Selection (MAS), marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS). So these molecular approaches like Marker-Assisted Selection (MAS), marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) can be effectively integrated into conventional breeding programs to develop abiotic stress responsive chickpea cultivars. Parental selection for these breeding programs can be done from already known abiotic stress resistant germplasm (as mentioned from various studies in previous section). Firstly, to imply these molecular approaches QTL mapping, their validation and fine mapping is done and then markers are developed. Subsequently, these markers, linked to enhanced agronomic performance and resistance to abiotic stress, are integrated into critical chickpea breeding programs. The following section of this article delves into the details of MAS and its utilization in the development of salinity-resistant chickpea cultivars.

Genetic approaches accelerate chickpea breeding through QTL mapping, marker-assisted breeding, and genomic selection, identifying superior genotypes and streamlining germplasm selection using molecular markers, also reducing multi-environmental phenotyping costs (Samineni *et al.,* 2016; Varshney *et al.,* 2014; Paul *et al.,* 2018; Roorkiwal *et al.,* 2018). Utilizing molecular markers to correlate genotypic and phenotypic traits is crucial for releasing well-adapted chickpea varieties, especially for less heritable abiotic stress tolerance traits (Gupta *et al.,* 2010). These strategies enable the dissection of complex traits into their constituent traits and the exploration of their genetic basis in chickpeas.

Salinity and drought tolerance in chickpea is a complex trait influenced by numerous genes and QTLs. Traditional genetic analyses are challenged by complex genotype-environment interactions, and limited phenotypic variation for drought salinity tolerance complicates chickpea breeding. QTL discovery, family-based mapping, and genomics locate genomic regions for salinity tolerance, yet only partially explain the trait's multigenic nature. Recent advancements identify genomic regions, offering candidate genes for marker-assisted breeding, shedding light on structural and functional attributes within essential pathways, and their validation across genotypes and environments. Marker-assisted selection (MAS) and marker-assisted backcrossing are powerful genetic approaches that facilitate the introgression of challenging-to-phenotype traits, such as root characteristics. These techniques not only expedite trait improvement but also minimize the linkage drag of genes with deleterious effects from wild donor parents (Varshney and Dubey, 2009). Marker-assisted selection (MAS) is particularly instrumental in chickpea breeding, where it enables the selection of traits such as productivity, disease resistance, abiotic stress tolerance, and quality through molecular markers. Comparison of conventional breeding approach and molecular assisted breeding has been presented in Fig.1. In the context of chickpea flowering control, specific genes (efl-1, efl-2, efl-3, and efl-4) and QTLs have been identified, influencing the timing of flowering, a crucial developmental stage (Kumar *et al.*, 2000). Additionally, genetic regions associated with essential chickpea traits have been well-documented across various conditions, offering an advantage for breeding early-maturing chickpea varieties capable of withstanding late-season abiotic stresses.

Notably, genomic region on linkage group 04 (CaLG04) in chickpea (*Cicer arietinum*), known as the "**QTL hotspot**" region, has stable QTLs for drought tolerance traits, holding promise for breeding efforts (Varshney *et al.,* 2014; Bharadwaj *et al.,* 2021; Barmukh *et al.,* 2022). Development of high yielding Fusarium wilt resistant cultivar by pyramiding of "genes" through marker-assisted backcrossing in chickpea. This QTL hotspot

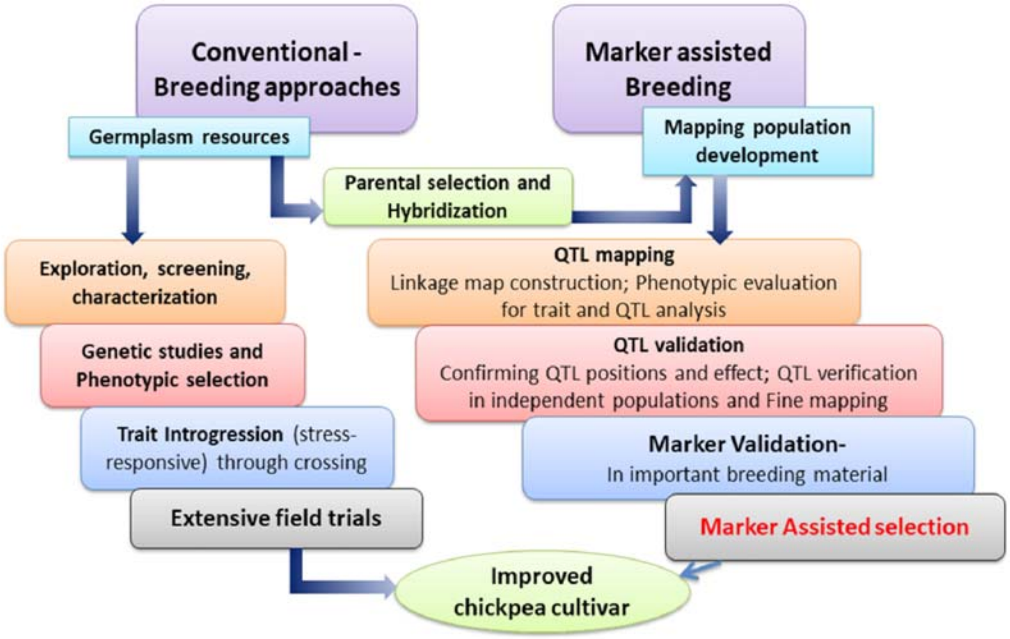

Fig. 1 Using MAS in conventional breeding for enhancement of abiotic stress tolerance

region was introgressed in three modern varieties of chickpea from donor parent ICC4858 by Bharadwaj *et al*., 2021 and has been presented by Bharadwaj *et al.* (2021). In addition to QTL analysis and genomics research, genotyping platforms play a pivotal role in expediting the breeding process by harnessing genome-level variation (Roorkiwal, 2018). Frequently, QTLs associated with correlated traits are found in close proximity, suggesting either pleiotropy or closely linked genes controlling these traits. The co-location of QTLs or QTL clusters offers an opportunity to simultaneously select for multiple traits, thereby enhancing the efficiency of chickpea breeding programs. Soren *et al.* (2020) identified two genomic regions that harbour QTLs for salinity tolerance in a ~3.3 Mb region on CaLG03 and ~0.1 Mb region on CaLG06 with major QTLs for yield and salinity tolerance. Molecular markers/genes associated with these major QTLs, after validation, will be useful to undertake marker-assisted breeding for developing better varieties with salinity tolerance. In a pioneering study, researchers discovered QTLs linked to seed yield under saline conditions on linkage group 3 in the late flowering group. Additionally, they identified a cluster of QTLs on linkage group 6, including one responsible for seed number, explaining 37% of the variation (Vadez *et al.,* 2012) This marks the first report of such QTLs for seed yield and its components in chickpea under salinity stress and these findings confirm the strong correlation between salinity tolerance in chickpea and successful reproduction under stress conditions. Various other studies report such QTLs which can be further utilized in QTL validation and fine mapping (Table 2). Following QTL identification and fine mapping, the next crucial step is marker development, enabling their early integration into breeding programs to expedite the breeding process and accurately select stress-tolerant genotypes.

Conclusions and Future Strategies

GWAS have emerged as powerful tools for unraveling the genetic makeup of complex traits in different taxonomical groups specially pulses that have a vital role in global food security. GWAS include the HTP (High Throughput Technology) of diverse pulse crop populations using enormous types of genetic markers over the genome. Statistical approaches are applied to identify significant associations between molecular markers and the traits such as yield, nutritional composition, seed size, seed shape, flowering time, root and pod traits under salt stress etc. By detecting the candidate genes after annotation, it can be further applied in molecular breeding techniques like MAS, genomic selection and other gene editing tools. However, it requires the validation of the recognized genetic loci for their use in future crop improvement programme. In conclusion, GWAS approach provides a radical way of looking at the complex genetic network underlying trait variation like salt stress in pulse crops. By combing the GWAS with conventional breeding approaches the full potential of pulses can be unlocked that can assist in maintenance of the food security.

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Appraisal of Groundwater Nitrate Accumulation in Faridkot and Firozpur District of South-Western Punjab

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Abstract

The study characterized nitrate concentration in groundwater of Faridkot and Firozpur district of Punjab and identified the pollution load of NO_3 as a result of the groundwater using field surveys and analyses of chemical constituents. The electrical conductivity ranged from 0.24-10.50 dS m^{-1} in Faridkot and 0.28-8.70 dS m^{-1} in Firozpur district with mean of 3.51 and 1.93 dS m^{-1} in Faridkot and Firozpur tehsil, respectively. The groundwater in Jaitu tehsil of Faridkot district and Firozpur tehsil of Firozpur district contains higher residual sodium carbonate with mean value of 2.85 and 3.57 meq L^{-1}. In Faridkot district the groundwater nitrate ranged from 0.09-1110 mg L^{-1} with average value of 502.16 mg L^{-1} in Faridkot tehsil followed by Kotkapura tehsil with average value of 52.78 mg L^{-1}. However, in Firozpur district it ranged from 2.27 -87.34 mg L^{-1} with average value of 39.77 mg L^{-1} in Firozpur tehsil followed by Guru Harsahai tehsils with average value of 25.11 mg L^{-1}. On an average in Faridkot district 10%, 65% and 25% water samples having safe (<10 mg L^{-1}), margin (10-150 mg L^{-1}) and unsafe (>150 mg L^{-1}) limits of nitrate. However, on an average 8.03% and 91.97% water samples recorded safe (<10 mg L^{-1}) and marginal (10-150 mg L^{-1}) value of-nitrate in Firozpur district.

Key words: Groundwater, Salinity, Sodicity, Nitrate accumulation

Introduction

High nitrate concentration in drinking water causes health problems such as blue baby syndrome (methemoglobinemia) in infants, thyroid disorders, spontaneous abortions and birth defects and cancer in adults (Almasri, 2007; Gupta *et al.,* 2008; Chang *et al.,* 2010). Next to surface water the groundwater is the most important water resource in meeting the domestic water requirement. Water contamination consists of physical, chemical or biological changes in water in a way that the water is not potable for human or usable for other purposes anymore. Nitrate is one of the most common contaminants in groundwater resources globally, and its presence can severely hamper clean water availability; high nitrate levels lead to limited reliance on such sources (Nolan *et al.,* 2015; Wagh *et al.,* 2020). The major sources of nitrate contamination in groundwater are due to decomposition of organic matters in soil, leaching of chemical fertilizers used in agriculture, human and animal waste, untreated effluent from industries rich in nitrogenous wastes and improper sewage disposal. Leaching of nitrate from different sources is more concerned in areas where there are shallow aquifers and where groundwater is the only source for potable water. In recent years, several studies have reported growing health risks of nitrate pollution and dramatic increases of nitrate concentration of groundwater in intensive agricultural in many parts of the world (Serio *et al.,* 2018; Soldatova *et al.,* 2018), especially in arid and semiarid areas with irrigated agriculture (Wang *et al.,* 2018; Adimalla and Li, 2019; Barakat *et al.,* 2019). However, little information is available on nitrate contamination of groundwater in Punjab, with concern to south-west zone. Therefore, the present study was conducted and the results of this study may be beneficial in establishing a groundwater protection plan to support the sustainable utilization of groundwater resources.

Materials and Methods

Location and weather of study area

The location map of the study area is shown in Fig. 1. The Faridkot (29°54′00″ and 34°54′00″ N;

Fig. 1 Location map of the surveyed area

74°15′00″ and 75°25′00″ E) and Firozpur districts (29°56′47″ and 31°0′7″ N; 72°52′4″ and 75°01′11″ E) are located in south-west part of the Punjab. Faridkot district shares common boundaries with Moga district in east, Firozpur district in North & West and Muktsar and Bathinda districts in south. However, Tarn Taran, Jalandhar and Kapurthala districts are located at north, Moga district in east, Faridkot district in south and Pakistan in the west side of the Firozpur district of the state. The climate of the districts can be classified as sub-tropical steppe, semi-arid and hot which is mainly dry except in rainy months and characterised by intensely hot summer and cold winter (Anonymous, 2022). The southwest monsoon sets in last week of June and withdraws towards end of September and contributes about 78-79% of annual rainfall. The Faridkot and Firozpur district receives about 449 mm and 389 mm annual rainfalls which are unevenly distributed over the area in 23-24 days.

Groundwater sampling and analytical techniques

A total of 541 and 466 GPS-based groundwater samples were collected form Faridkot and Firozpur district, respectively. The samples from running tube wells were collected in pre-cleaned polyethylene bottles, sealed, labeled and brought to the laboratory for analysis. Samples were kept at 4 °C until the analysis was completed. Standard analytical procedures, as suggested by APHA (2005) for the analysis of groundwater samples, were followed throughout. Electrical conductivity (EC) and pH were analyzed using digital meters. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined using EDTA titration. Chloride (Cl^-) was determined by standard $AgNO_3$ titration. Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) were determined by titration with HCl. Sodium (Na^+) and potassium (K^+) were measured by flame photometry. Sodium adsorptionratio (SAR) and residual sodium carbonate (RSC) in water samples were calculated as described by the following equation:

$$\text{SAR (m moL}^{-1})^{1/2} = \frac{Na^+}{\sqrt{(Ca^{+2} + Mg^{+2})/2}}$$

$$\text{RSC (meq L}^{-1}) = (CO_3^{2-} + HCO_3^-) - (Ca^{+2} + Mg^{+2})$$

(All values of cations and anions are in meq L^{-1})

Solubilized nitrate was determined potentiometrically using a nitrate ion-selective electrode (ISE) in conjunction with a double-junction reference electrode meter (OAKTON-Ion 2700) equipped with an ISE meter capable of being calibrated directly in terms of nitrate concentration (mg L^{-1}). The analytical results of nitrate in water have been categorized as safe, margin and unsafe based on nitrate content as <10 mg L^{-1}, 10-150 mg $L^{-1}$and >150 mg L^{-1}, respectively.

Results and Discussion

Chemical properties of groundwater

Different chemical parameters of ground water in tehsils of Faridkot and Firozpur district were presented in Table 1. The electrical conductivity (EC) of water ranged between 0.46-9.50 dS m^{-1} with mean 3.51 dS m^{-1}, 0.24-10.50 dS m^{-1} with mean 2.85 dS m^{-1} and 0.50-5.50 dS m^{-1} with mean 2.43 dS m^{-1} in Faridkot, Jaitu and Kotkapura tehsils, respectively in Faridkot district. Whereas, the water electrical conductivity (EC) ranged between 1.0-8.7 dS m^{-1} with mean 3.16 dS m^{-1}, 0.60-4.8 dS m^{-1} with mean 1.93 dS m^{-1} and 0.28-4.50 dS m^{-1} with mean 1.79 dS m^{-1} in Firozpur, Guru Harsahai and Zira tehsils, respectively, in Firozpur district.

The chemistry of groundwater and the main ionic species that are present in it are likely being impacted by rock-water interaction, as evidenced by a greater TDS value (Kumar *et al.,* 2020). The results are confirm with the findings of Katiyar *et al.* (2014), Yadav and Sekhon (2016), Kaur *et al.* (2017), Yadav *et al.* (2018) and Yadav and Kumar (2021), who reported the dominance of sodium and chloride ions in irrigation water in arid and semi-arid regions. In Faridkot district, Jaitu tehsil contain higher RSC (2.85 meq L^{-1}) followed by Kotkapura (2.8 meq L^{-1}) and Faridkot tehsil (1.66 meq L^{-1}). Whereas, maximum Ca^{+2} + Mg^{+2} was reported in Faridkot tehsil and minimum average value is recorded in Kotkapura tehsil. However, Firozpur tehsil contain higher RSC (3.57 meq L^{-1}) followed by Guru Harsahai (2.69 meq L^{-1}) and Zera tehsil (1.97 meq L^{-1}) in Firozpur district. The majority of groundwater in Faridkot has high salt content and high RSC (Sharma *et al.,* 2017). Similar results were also reported by Yadav and Sekhon (2016), Kaur *et al.* (2017), Yadav *et al.* (2018) and Yadav and Kumar (2021). Among the anions, chloride was dominant anion ranging from 0.40 to 22.20 meq L^{-1} in Faridkot and 0.80 to 19.0 meq L^{-1} in Firozpur district followed by bicarbonate (0.10-13.0 meq L^{-1}) and carbonate (0.0

Table 1. Range and average of different chemical constituents of ground water in Faridkot and Firozpur district of Punjab

District	Faridkot (541)			Firozpur (466)		
Blocks	Faridkot (164)	Jaitu (194)	Kotkapura (183)	Firozpur (170)	Guru Harsahai (120)	Zira (176)
Parameters	Range	Range	Range	Range	Range	Range
pH	7.2-10.2	7.1-9.2	7.3-9.3	7.1-10.2	7.1-9.5	7.6-10.1
	(8.6)	(7.9)	(8.2)	(8.4)	(7.9)	(8.7)
EC	0.5-9.5	0.2-10.5	0.5-5.5	1.0-8.7	0.6-4.8	0.3-4.5
(dS m^{-1})	(3.5)	(2.6)	(2.4)	(3.2)	(1.9)	(1.8)
Ca^{+2} +Mg^{+2}	2.3-68.0	1.0-18.1	0.8-10.5	0.8-9.9	1.5-10.2	0.7-10.2
(meq L^{-1})	(6.8)	(5.6)	(3.9)	(4.6)	(5.7)	(4.3)
Cl^{-1}	0.5-18.0	0.4-22.2	1.0-9.0	0.8-19.0	1.0-16	0.8-15.6
(meq L^{-1})	(6.1)	(5.6)	(3.7)	(5.9)	(3.0)	(3.7)
CO_3^{-2}	0.0-0.6	0.0-0.8	0.0-0.6	0.0-1.0	0.0-0.3	0.0-0.9
(meq L^{-1})	(0.3)	(0.3)	(0.3)	(0.5)	(0.2)	(0.3)
HCO_3^-	1.2-11.0	1.6-13.0	0.1-12.2	2.0-11.6	1.2-13.6	1.0-9.0
(meq L^{-1})	(5.2)	(6.4)	(6.1)	(7.0)	(6.4)	(4.3)
RSC	0.0-4.9	0.0-8.0	0.0-8.20	0.0-8.0	0.0-12.0	0.0-4.6
(meq L^{-1})	(1.7)	(2.9)	(2.8)	(3.6)	(2.7)	(2.0)
SAR	0.6-39.7	1.1-58.3	3.2-47.0	0.8-19.9	0.3-8.6	0.5-14.0
(m mol L^{-1})	(3.3)	(7.4)	(10.6)	(7.7)	(2.8)	(5.6)
K	0.12-6.57	0.2-13.49	0.31-9.03	0.02-4.03	0.27-0.66	0.01-2.35
(me L^{-1})	(1.37)	(1.22)	(1.04)	(1.01)	(0.041)	(0.64)
Na	1.3-46.7	1.4-131.6	6.3-70.5	0.8-25.6	0.6-10.4	0.9-21.4
(me L^{-1})	(5.58)	(12.3)	(14.0)	(10.4)	(4.03)	(7.5)

*Values in parenthesis denote the number of samples and the average value, respectively.

Table 2. Range of nitrate (mg L^{-1}) in Faridkot and in Firozpur district of Punjab

District	Faridkot			Firozpur		
Blocks	Faridkot	Jaitu	Kotkapura	Firozpur	Guru Harsahai	Zira
Minimum	0.09	1.68	6.60	2.48	2.27	2.46
Maximum	1410	125.15	176.0	87.34	67.8	62.20
Average	502.16	27.04	52.78	39.77	25.11	19.89

to 0.80 meq L^{-1}) in Faridkot and bicarbonate (0.10-13.6 meq L^{-1}) and carbonate (0.0 to 0.90 meq L^{-1}) in Firozpur districts. Earlier, Yadav and Kumar (2021) also reported similar results in groundwater of Bathinda and Mansa districts in southern part of Punjab.

Nitrate accumulation and distribution

Accumulation of nitrate in ground water of Faridkot and Firozpur district was presented in Table 2. The ground water nitrate ranged from 0.09-1110 mg L^{-1} with mean 502.16 mg L^{-1}, from 1.68-125.15 mg L^{-1} with mean 27.04 mg L^{-1} and from 6.60-176.0 mg L^{-1} with mean 52.78 mg L^{-1} in Faridkot, Jaitu and Kotkapura tehsils, respectively in Faridkot district. Whereas, in Firozpur district the nitrate content ranged from 2.84-87.34 mg L^{-1} with mean 39.77 mg L^{-1}, from 2.27-67.8 mg L^{-1} with mean 25.11 mg L^{-1} and from 2.46-62.20 mg L^{-1} with mean 19.89 mg L^{-1} in Firozpur, Guru Harsahai and Zira tehsils, respectively. The maximum nitrate variation was reported in Faridkot tehsils with average of 502.16 mg L^{-1} followed by Kotkapura tehsil with average of 52.78 mg L^{-1}.

Likewise, the maximum nitrate variation are reported in Firozpur tehsil with average of 39.77mg L^{-1} followed by Guru Harsahai tehsil with average of 25.11mg L^{-1}. The table 2 also reveal that the ground water in Jaitu tehsil of Faridkot district and Zira tehsil of Firozpur district contain low amount of nitrate (27.04 mg L^{-1}) and (19.89 mg L^{-1}) respectively, as compared to other tehsils of their respective districts. The probable sources of nitrate contamination of groundwater are through excessive application of fertilizers, bacterial nitrification of organic nitrogen, and seepage from animal and human wastes and atmospheric inputs. In Punjab state, nitrate in water samples varies from <0.20 mg L^{-1} (traces) to 617 mg L^{-1}. A considerable area of the southern and south-western part of the state have nitrate concentration exceeding 45 mg L^{-1} in ground waters of different blocks of Firozpur, Faridkot and Bathinda districts (Sharma *et al.,* 2016). Furthermore, quite a significant number of water samples from, Faridkot, Firozpur, Muktsar and Sahibzadad Ajit Singh Nagar districts were found to have nitrate above 100 mg L^{-1} (Anonymous, 2022). Nitrate concentration above permissible limit (> 45 mg L^{-1}) were found in different villages of Faridkot district, such as Sukhanwala (46 mg L^{-1}), Shersinghwala (65 mg L^{-1}), Wara Daraka (66 mg L^{-1}), Dhilwan Kalan (72 mg L^{-1}), Nathuwala (79 mg L^{-1}), Chand Bhaja (79 mg L^{-1}), Bir Sikhanwala (89 mg L^{-1}), Nangal (104 mg L^{-1}), Tehna (118 mg L^{-1}), Dal Singhwala (136 mg L^{-1}), Killi (140 mg L^{-1}), and Kotkapura (144 mg L^{-1} ; (Anonymous, 2017).

The percent distribution of nitrate (mg L^{-1}) in different blocks of Faridkot and Firozpur district of Punjab were presented in Fig. 2. It showed that the most of water samples (79%) collected from Faridkot tehsil have higher amount of fluoride, which makes it unsuitable for use. However, in Jaitu tehsil the ground water having safe to marginal nitrate concentration and only 4% sample were found unsafe limit (>150 mg L^{-1}) of nitrate in ground water.

On an average in Faridkot district 10%, 65% and 25% water samples having safe (<10 mg L^{-1}), margin (10-150 mg L^{-1}) and unsafe (>150 mg L^{-1}) limits of nitrate. About 2.35 %, 5.83 % and 15.91% water samples collected from Firozpur, Guru Harsahai and Zira tehsil showed low content (<10.0 mg L^{-1}) of nitrate and were suitable for safe use. However, 97.65 %, 94.17 % and 84.09 % water samples collected from Firozpur, Guru Harsahai and Zira tehsil showed marginal amount (10.0-150 mg L^{-1}) of nitrate. On an average in Firozpur

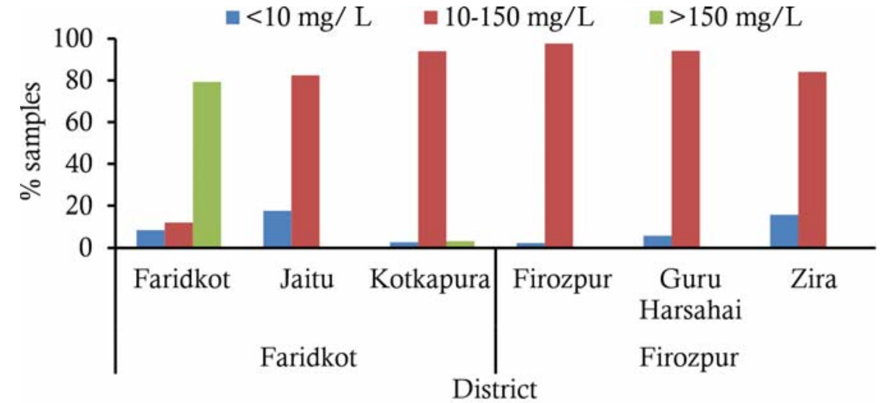

Fig. 2 Percent distribution of nitrate (mg L^{-1}) in different blocks of Faridkot and Firozpur district

district 8.03% and 91.97 water samples having safe (<10 mg L^{-1}) and marginal (10-150 mg L^{-1}) amount of nitrate. The different nitrate concentration in the area may be due to different local agricultural practices in a particular area. In rural areas, NO_3–N derived from livestock waste and fertilizers used in agricultural practices enters groundwater, which eventually affects the eutrophication (Lee and Choi, 2010; Pastén-Zapata *et al.,* 2014).

Conclusions

It is concluded from the present study that maximum mean EC (3.51 dS m^{-1}) was reported in Faridkot tehsil, whereas, maximum RSC (2.85 meq L^{-1}) was reported in Jaitu tehsil of Faridkot destrict. Similarly, higher mean EC (3.16 dS m^{-1}) and RSC (3.57 meq L^{-1}) was reported in Firozpur tehsil of Firozpur district. The higher mean of nitrate (502.16 mg L^{-1} and 39.77 mg L^{-1}) were reported in Faridkot and Firozpur thesil of their respective districts. The results also shown that (65%) water samples in Faridkot district and (91.97%) water samples in Firozpur district having marginal (10-150 mg L^{-1}) amount of nitrate. However, 25 % water samples in Faridkot district were reported beyond unsafe limits (>150 mg L^{-1}) which is matter of concern and needs planning..

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Assessment and Mapping of Groundwater Quality and its Impact on Soil Properties of Sohna Block in Gurugram District of Haryana State

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Abstract

In Sohna block of Gurugram district, a study was conducted to evaluate the quality of the groundwater and determine how it affected the soil qualities. Using a handheld GPS, 79 groundwater samples and 48 soil samples overall were taken from two sites from each category of water, four depths each, in the Sohna block of Gurugram districts. The pH, EC, SAR and RSC ranged from 7.31 - 8.28, 0.75-8.42 (dS m^{-1}), 6.38 – 19.00 (mmol $l^{-1})^{1/2}$ and nil to 4.20 (me l^{-1}) in the block. The anions contents in water followed the pattern $Cl^- > SO_4^{2-} > HCO_3^- > NO_3^- > CO_3^{2-}$ whereas the cations were found to be in the following order: $Na^+ > Mg^{2+} > Ca^{2+} > K^+$. The village Isaka had the lowest saturation percentage (28.72%) and the village Abhepur had the highest saturation percentage (35.36%). The settlement of Sohna Rural in the Sohna block has the lowest EC (1.22 dS m^{-1}) of saturated extract. The village of Alipur in Sohna block had the highest EC of saturated extract (8.20 dSm^{-1}) whereas Sanp Ki Nagli (7.66) and Darbaripur (8.47) were the villages in Sohna block with the lowest and highest pH values, respectively. In whole of the block, Cl^- and Na^+ of the soil saturation extract were significantly and positively correlated with EC, Cl^- and EC, Na^+ of groundwater used for irrigation, respectively.

Key words: Anions, Cations, Groundwater quality, RSC, SAR, Saturation percentage

Introduction

Agricultural as well as human utilization of groundwater is based on quality of groundwater for irrigation use; water quality can be determined by assessment of concentration and composition of dissolved salts in water. Irrigation as well as human utilization and groundwater quality outcome on soil properties is largely site specific with possible variations with time due to climatic conditions, residence time of water within the aquifer materials and human activities (Oladeji *et al.,* 2012; Ewusi *et al.*, 2013). According to CGWB (2009) estimates, overall water use in Gurugram district has exceeded the available recharge and accordingly the district has been categorized as over exploited. Current gross ground water utilization of district is 53927 ha-m which exceed the net annual ground water availability of the district 23261ha-m (Anonymous, 2021). Quality of water for irrigation is correlated to its impact on soils and crops as well as its management. The use of good quality irrigation water can help in production of crops of good quality with high yield (Islam and Shamsad, 2009). Agriculture needs to be sustainable, thus soil and water management, monitoring, and use are essential. In arid and semiarid locations, over drafting and deteriorating ground water quality are seriously harming crop productivity (Boumans *et al.*, 1988).

Although Haryana state is one among the major state in terms of agricultural production of the country but due to amplified use of chemical fertilizers and pesticides, state is facing various physical and ecological problems such as contamination of ground water and surface water with nutrients and toxic substances. The soil salinity and alkalinity problem is continuously increasing in state as well as in study area because of dissolved salts in water and its improper utilization.

Now a day due to various development activities, over-exploitation of water resources have increased to great extent due to which, quality as well as quantity of water available for use is affected severely. Because of pavement in urban areas, groundwater recharge is reducing and groundwater withdrawal is increasing affecting its availability (Kumar *et al.*, 2015). Limited work at block level has been studied so far in reference to quality of soil and water. Therefore, an appraisal for the quality of irrigation water is essential for sound irrigation planning so as to assess any possibility of development of secondary salinization/sodification in Haryana. So, keeping in view the above facts, the present studies were proposed.

Materials and Methods

Area and location

Gurugram district of Haryana is located between 27°39′00″ North and 28°32′25″ North latitudes and between 76°39′30″ East and 77°20′45″ East longitudes (Fig. 1). Gurugram Plain and Sohna Undulating Plain with Aravalli Hills are the two main sub-parts of Gurugram District from a physiographic perspective. Few crops were grown in the undulating plains of Sohna because of the poor soil coverage and the rough surface caused by rocks.

Soil and climate

The majority of the Gurugram district's soils are classified as Ochreptps kinds, while Orthids-Fluvents and Ochrepts Ustrets-Ustalfs types are found in the district's central and south-western regions. The Gurugram district has sandy to sandy-loam type of soil (DCO Haryana, 2011). The Gurugram district has sub-tropical continental monsoon climate. The highest daily mean temperature of 40.2° C occurs in month of May. The average rainfall of Gurugram district during year 2018 was 386.7 mm (IMD, 2018).

Collection of water samples

A total of 79 water samples from tube wells in the Sohna block of the district of Gurugram were taken in 2018–19 to evaluate the quality of the groundwater and to locate the exact site of sampling, longitude and latitude of locations were recorded using hand held GPS.

Processing and analysis of water samples

Firstly, filtration of water samples was carried out in laboratory and then to avoid microbial

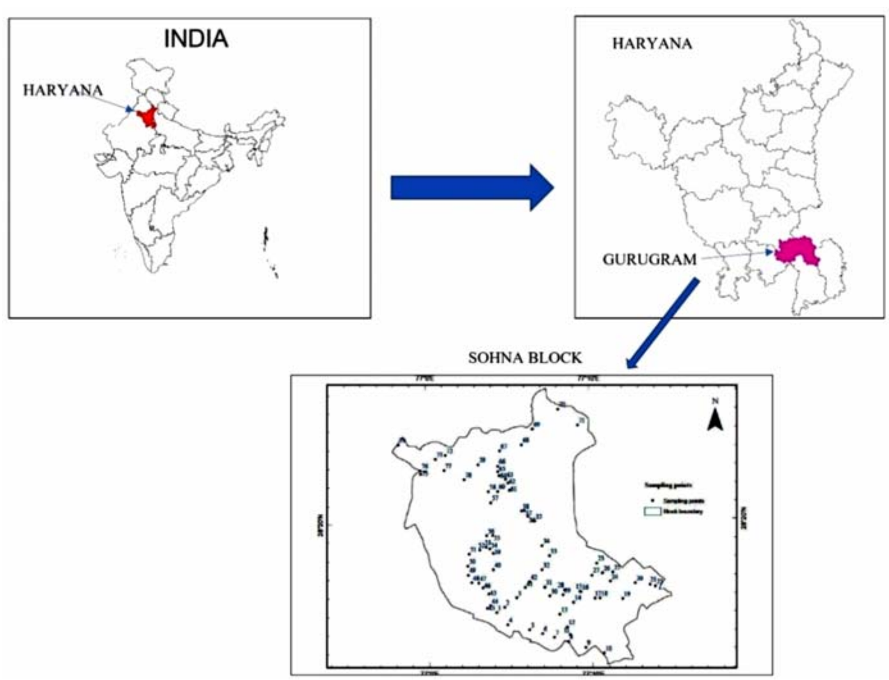

Fig. 1 Location map of the study area

contamination, one or two drops of toluene were added to the bottles. After processing, ground water samples were analysed for pH and EC using pH meter and conductivity meter. Among soluble cations, calcium and magnesium were determined using Versante titration method (Diehl *et al*., 1950), sodium and potassium using flame photometer and the anions (CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-} and NO_3^-) by their standard analytical procedures. Sodium adsorption ratio (SAR) and residual sodium carbonate (RSC) were calculated by using the following equations given by Richards (1954) and Raghunath (1987), respectively for the purpose of classification of water quality:

$$SAR\ (mmol\ l^{-1})^{1/2} = Na^+ / [(Ca^{2+} + Mg^{2+})/2]^{0.5}$$

$$RSC\ (me\ l^{-1}) = [CO_3^{-2} + HCO_3^-] - [Ca^{+2} + Mg^{+2}]$$

Characterization of irrigation water

Water quality has been characterized as per Tiwari and Sharma (1989) and has been shown in Table 1.

To study the spatial distribution of quality parameters in the whole block, a spatial variability map was prepared by using ArcGIS (Arc Map 9.3 software) through the interpolation of the available data from the sampling points.

Determination of soil properties

Soil samples were collected from 0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm depth of fields with the help of auger depending upon the results of ground water quality for fulfilling the purpose of determination of impact of groundwater quality on various soil properties. Soil samples were collected from same field from where groundwater samples of different categories were collected. A total of 10 sites were selected, 2 from each category of water quality for sampling of soil samples in Sohna block on the basis of results of water quality analysis. Take 2 mm sieved soil sample in a beaker and add distilled water to it after it stirring with spatula was done for the purpose of preparation of soil saturation paste.

This method of preparation of soil saturation paste was explained by US Salinity Laboratory Staff (1954). Then saturation extract collected from soil saturation paste was used for analysis of various soil properties such as, soluble cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), anions (CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}), $CaCO_3$ and saturation percentage as per standard procedures as described for water analysis (Richards, 1954). Soil samples were also analyzed for EC (1:2) and pH (1:2) by using conductivity meter and pH meter consisting of glass electrode in 1:2 (soil: water) suspension at room temperature 25°C (Jackson, 1973).

Statistical methods

Karl Pearson method of correlation was used for computation of correlation coefficient among various parameters of ground water and soil extracts (Panse and Sukhatme, 1954).

Results

Quality of irrigation water

The values of different water quality parameters were depicted in Table 2. The result of analysis of ground water samples of Sohna block showed

Table 1. Irrigation water quality classification criteria

S.N.	Quality of water	Class	Quality parameter		
			EC (dSm^{-1})	SAR ($mmol\ l^{-1})^{1/2}$	RSC ($me\ l^{-1}$)
1	Good	A	<2	<10	<2.5
2	Saline	B	-	-	-
	Marginally saline	B_1	2-4	<10	<2.5
	Saline	B_2	>4	<10	<2.5
	High SAR saline	B_3	>4	>10	<2.5
3	Alkali water	C	-	-	-
	Marginally alkali	C_1	<2	<10	2.5-4
	Alkali	C_2	<2	<10	>4
	Highly alkali	C_3	Variable	>10	>4

that pH, EC, SAR and RSC were 7.31 - 8.28, 0.75- 8.42 (dS m^{-1}), 6.68 – 19.00 (mmol l^{-1})1/2 and nil to 4.20 (me l^{-1}), respectively. The lowest value of pH (7.31), EC (0.75 dSm^{-1}) and SAR [6.68 (mmol L^{-1})1/2] found in village Kuliaka, Rahaka and Sanp Ki Nagli, respectively while the highest value of pH (8.28), EC (8.42 dSm-1) and SAR [19.00 (mmol $l^{-1})^{1/2}$] found in village Darbaripur, Alipur and Abhepur, respectively. The cations were found to be in the order of $Na^+ > Mg^{2+} > Ca^{2+} > K^+$ and ranged from 5.95 to 43.54 meq l^{-1}, 0.60 to 28.60 meq l^{-1}, 0.20 to 9.60 meq l^{-1}, 0.11 to 0.36 meq l^{-1} respectively while anions followed in order of $Cl^- > SO_4^{2-} > HCO_3^- > NO_3^- > CO_3^{2-}$ and varied from 1.60 to 57.40 meq l^{-1}, 0.30 to 17.80 meq l^{-1}, 1.10 to 7.20 meq l^{-1}, nil to 1.05 meq l^{-1}, nil to 0.90 meq l^{-1} respectively. The spatial variability of EC, pH, RSC and SAR is represented by Figs. 4, 5, 6 and 7, respectively.

AICRP (1989) classification of groundwater quality of Sohna block

The water samples were classified based on the criteria given by All India Coordinated Research Project (AICRP) on "Management of Salt Affected Soils and Use of Saline Water in Agriculture" (1989) in which water quality has been grouped into three categories such as good, saline and alkali. This classification is based upon EC, SAR and RSC Parameters. Based on the limits of various parameters, two poor quality water classes have been further classified each into 3 subclasses. The data pertaining to classification of groundwater quality as per AICRP (1989) criteria according to which 29 groundwater samples were recorded under good (A) category, 25 under marginally saline (B1) category, 3 under saline (B2) category, 15 under high SAR saline (B3) category, 3 under marginally alkali (C1) category and 4 under high alkali (C3) category is presented in table 5. Fig. 2 and 3 represents the distribution percentage of water samples and spatial variability of groundwater quality in different water quality categories of Sohna block. According to AICRP (1989) criteria of categorization of groundwater quality, the quality of water of village Isaka and Sanp Ki Nagli (Good quality), Hajipur and Kuliaka (Marginally saline),

Table 2. Chemical composition of tube well water used for irrigation in different villages of Sohna block of Gurugram district

Properties	Villages of Sohna block											
	Isaka	Sanp ki Nagli	Hajipur	Kuliaka	Khroda	Alipur	Sanchauli	Abhepur	Sohna Rural	Rahaka	Darbaripur	Palra
EC	1.51	1.15	2.8	2.52	4.35	8.42	4.25	5.46	0.76	0.75	1.32	1.3
pH	7.47	7.39	8.17	7.31	7.49	7.47	7.76	7.46	7.55	7.60	8.28	8.27
CO_3^{-2}	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.90	0.10	0.20
HCO_3^-	1.40	1.10	1.60	2.20	1.90	7.20	7.10	4.25	3.90	2.70	4.90	4.90
Cl^-	10.30	7.60	20.40	16.30	31.25	57.40	25.15	34.75	1.60	1.90	6.10	5.40
SO_4^{-2}	1.70	1.70	4.25	6.15	9.60	17.80	8.55	13.60	0.30	0.70	0.80	1.15
NO_3^-	Nil	Nil	0.21	0.28	0.26	1.05	0.53	0.53	Nil	Nil	Nil	0.23
Ca^{+2}	1.00	0.80	2.20	2.00	5.00	9.60	3.00	2.25	0.30	0.25	0.20	0.30
Mg^{+2}	2.85	2.00	6.30	5.80	14.70	28.60	11.00	8.25	1.00	0.65	0.60	0.70
Na^+	10.82	7.90	19.15	17.21	21.07	40.06	28.45	43.54	5.95	6.24	11.75	11.35
K^+	0.40	0.17	0.36	0.19	0.28	0.15	0.14	0.13	0.11	0.21	0.63	0.44
RSC	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2.60	2.70	4.20	4.10
SAR	7.80	6.68	9.29	8.71	6.71	9.17	10.75	19.00	7.38	9.30	18.58	16.05
Category AICRP	A	A	B_1	B_1	B_2	B_2	B_3	B_3	C_3	C_1	C_3	C_3

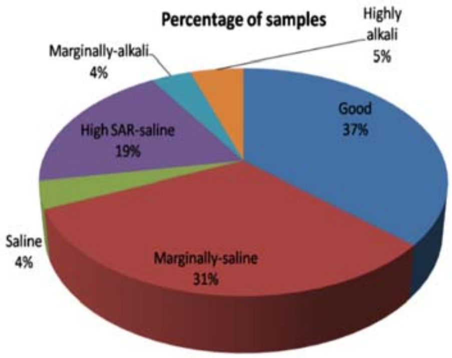

Fig. 2 Water quality distribution as per AICRP (1989)

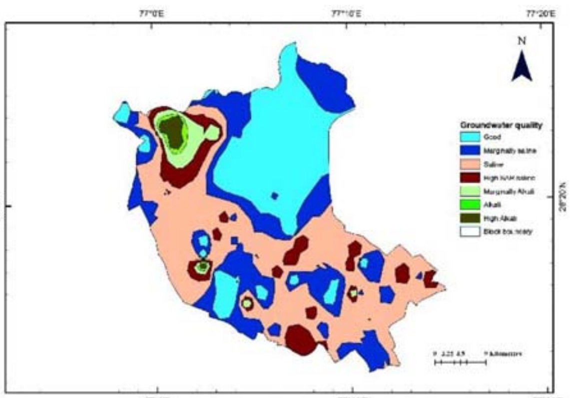

Fig. 3 Spatial variability of groundwater quality of Sohna block

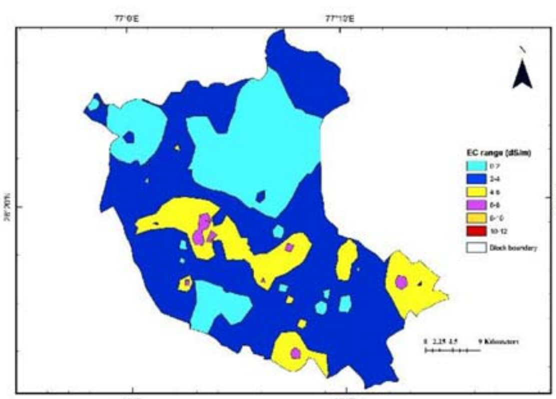

Fig. 4 Spatial variability in EC of groundwater of Sohna block

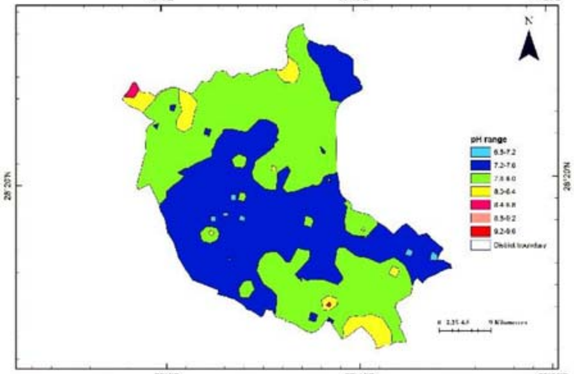

Fig. 5 Spatial variability in pH of groundwater of Sohna block

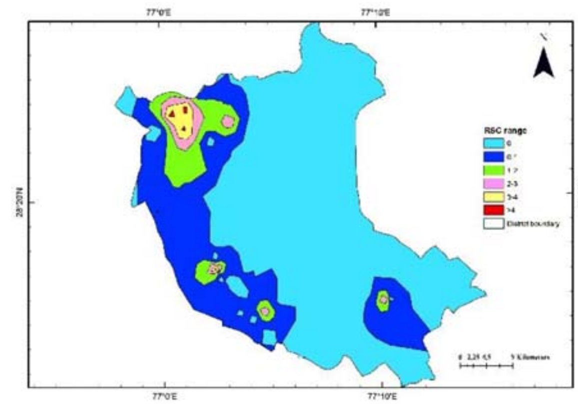

Fig. 6 Spatial variability in RSC of groundwater of Sohna block

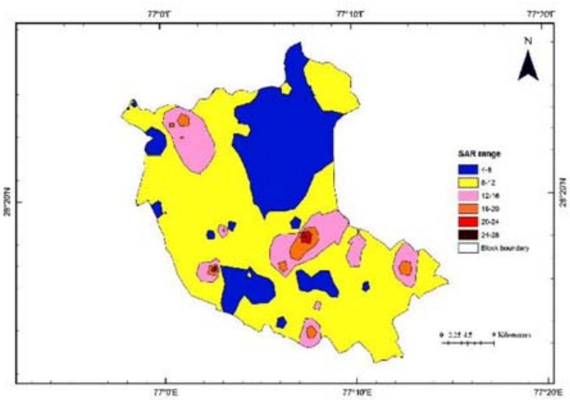

Fig. 7 Spatial variability in SAR of groundwater of Sohna block

Khroda and Alipur (Saline), Sanchauli and Abhepur (High SAR value water), Sohna Rural (Highly alkali), Rahaka (Marginally Alkali), Darbaripur and Palra (High alkali water) were found.

Chemical properties of soil

The data related to chemical properties of soil were illustrated in Table 3. In the village of Sohna Rural (1.22 dS m^{-1}) of the Sohna block, where water from the slightly alkali (C1) category was used, the mean lowest EC of saturation extract was found. When saline (B2) category water was used, the village of Alipur in Sohna block recorded the mean highest EC of saturation extract (8.20 dS m^{-1}). Village Kuliaka and Darbaripur were found to have the mean lowest and mean highest pH values, 7.44 and 8.47, respectively. Average accumulation of cations and anions was found to be in the following order: $Na^{+}>Ca^{2+}>Mg^{2+}>K^{+}$ and $Cl^{-}> HCO_3^{-}> SO_4^{2-} >CO_3^{2-}$, respectively.

Table 3. Impact of groundwater quality on soil properties of different villages of Sohna block of Gurugram district

Properties	Villages of Sohna block											
	Isaka	Sanp ki Nagli	Hajipur	Kuliaka	Khroda	Alipur	Sanchauli	Abhepur	Sohna Rural	Rahaka	Darbaripur	Palra
EC	1.96	1.48	3.51	4.07	5.49	8.20	5.20	6.38	1.22	1.23	2.27	2.71
pH	7.68	7.66	8.13	7.44	7.73	7.83	7.70	7.69	8.11	7.59	8.47	8.21
CO_3^{-2}	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.85	0.75	0.84
HCO_3^-	4.20	3.25	9.58	5.95	13.64	12.59	12.80	18.10	3.81	6.20	8.29	9.50
Cl^-	11.89	9.69	19.29	28.58	31.50	54.43	30.06	31.76	6.23	4.49	10.23	13.45
SO_4^{-2}	1.80	1.69	5.40	5.56	7.69	12.78	7.73	11.09	1.69	0.53	3.16	2.48
Ca^{+2}	3.70	2.56	5.99	7.81	15.90	22.14	7.26	14.04	1.20	1.54	1.25	3.05
Mg^{+2}	2.60	2.05	6.79	8.09	15.00	21.73	9.81	6.64	1.53	1.10	2.00	4.10
Na^+	12.03	9.32	21.04	22.52	21.88	36.91	31.70	40.85	8.20	8.52	17.33	16.36
K^+	1.21	0.47	1.05	1.75	0.91	0.74	0.79	1.30	1.06	1.08	1.85	2.18
Saturation (%)	28.72	32.89	30.54	31.33	34.36	31.21	33.99	35.36	32.45	34.22	32.21	31.62
$CaCO_3$ (%)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Statistical analysis of different water quality parameters and soil saturation extract of Sohna block

The correlation coefficient between groundwater and soil parameters of Sohna block is presented in table 4. The correlation coefficient indicated that electrical conductivity (EC) and pH of soil saturation extract was significantly and positively correlated with EC (0.977), Cl^- (0.969), Na^+ (0.944) and pH (0.844) of groundwater used for irrigation, respectively. Likewise, Cl^- and Na^+ of soil were significantly and positively correlated with EC (0.968), Cl^- (0.968) and EC (0.895), Na^+ (0.982) of groundwater used for irrigation, respectively.

Discussion

Quality of ground water

The electrical conductivity (EC) of groundwater samples collected from Sohna block varied between 0.75 to 8.42 dS m^{-1} with average EC of 2.89 dS m^{-1} and the pH value of groundwater samples varied between 7.31 to 8.28 with average value of 7.61. According to the pH value, water of Sohna block can be classified as neutral to alkaline in nature. Higher value of pH could be due to high concentration of ions such as Sodium and bicarbonates. Bicarbonates produce hydroxyl ion which is reason for increase or decrease in pH value. Mukesh (2003), Deshmukh (2012), Kumar (2015), Mandal *et al.* (2016) and Pal (2017) obtained similar results. Sodium was recorded as governing cation among all the cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) examined. Chemical decomposition of feldspathoid, feldspar and iron and magnesium minerals led to occurrence of sodium ion concentration in groundwater of Sohna block. Surface soil's salt leaching due to irrigation water or rain water could also be reason behind the dominance of Na^+ and Cl^- in the groundwater. It was found that concentration of all cations analyzed increased with increase in value of EC in the block. It was also observed that degree of increase in sodium and magnesium concentration was much higher than other anions. Results of the study were in line with Shahid *et al.* (2008) and Rathi *et al.* (2018). Likewise chemical decomposition of minerals rich in calcium like

Table 4. Correlation coefficient between groundwater and soil parameters of Sohna block of Gurugram district

	EC_{iw}	EC (Soil)	pH (water)	pH (soil)	Cl^- (water)	Cl^- (Soil)	Na^+ (water)	Na^+ (Soil)
EC_{iW}	1	-	-	-	-	-	-	-
EC (Soil)	.977**	1	-	-	-	-	-	-
pH (water)	-.268	-.216	1	-	-	-	-	-
pH (soil)	-.226	-.226	.844**	1	-	-	-	-
Cl^- (water)	.994**	.969**	-.287	-.243	1	-	-	-
Cl^- (soil)	.968**	.977**	-.295	-.284	.968**	1	-	-
Na^+(water)	.933**	.944**	-.200	-.210	.910**	.879**	1	-
Na^+ (Soil)	.895**	.937**	-.106	-.168	.865**	.871**	.982**	1

** Correlation is significant at the 0.01 level of significance

*Correlation is significant at the 0.05 level of significance

Table 5. AICRP (1989) classification of groundwater quality of Sohna block of Gurugram district

Water quality	Class	Number of samples	Percentage
Good	A	29	36.70
Saline waters	B	-	-
Marginally-saline	B_1	25	31.65
Saline	B_2	3	3.80
High SAR-saline	B_3	15	18.99
Alkali waters	C	-	-
Marginally-alkali	C_1	3	3.80
Alkali	C_2	0	0.00
Highly alkali	C_3	4	5.06
Total		79	100.00

pyroxenes, feldspar and amphiboles led to presence of calcium ion concentration in groundwater. In case of anions, chloride was recorded as governing anion among all the anions (CO_3^{2-}, HCO_3^-, Cl, SO_4^{2-} and NO_3^-) analyzed. After that order of dominance of anions was sulphate, bicarbonate, nitrate and carbonate in groundwater of the block. It was observed that HCO_3^-, Cl^-, SO_4^{2-} and NO_3^- concentration have positive and significant correlation with EC of groundwater samples. In all the EC classes, sodium and chloride were the major cation and anion, respectively. Sharma, (1998), Ramprakash *et al.* (2020), Rajpaul *et al.* (2014), Sanjay *et al.* (2016) confirmed similar results. In contrast to bicarbonates concentration, concentration of carbonates was very low in groundwater. Dissolution of carbonic acid of aquifers and weathering of carbonates can be the reason for presence of CO_3^{2-} and HCO_3^- ions in water samples. Oxidation of pyrite, sulphur in igneous rocks, merasite, sulpharite and solution of other sulphur bearing minerals results in presence of sulphate ion concentration in groundwater. Findings are in agreement of results recorded by Kumaresan and Riyazuddin (2005), Pradhan *et al.* (2011) and Rahman *et al.* (2013). Industrialization, urbanization and fertilizers and chemicals application led to occurrence of nitrate concentration in groundwater of Sohna block of Gurugram district. Shahid (2004), Jitender (2006) and Kumar and Kumar (2015) also found nitrate concentration in ground waters of Julana block of Haryana, Karnal block of Karnal, Haryana and Kisanganj district of Bihar, Rewari/Bawal block of Haryana, Kaithal district of Haryana and Firozpur-Jhirka and Punhana block of Haryana, respectively.

The SAR value of groundwater sample taken from Sohna block of Gurugram district varied between 4.22 to 24.38 with average value of 9.64 $(mmol^{-1})^{1/2}$. Combination of Sodium with carbonates and bicarbonates results in sodicity hazard due to removal of exchangeable calcium and magnesium from soil solution. Sodic condition due to decrease in exchangeable calcium and calcium precipitation results from usage of water with high SAR value. With increase in concentration of sodium ion, increase in SAR value of groundwater samples was also observed by Singh *et al.* (2011) and Kumar (2015). Residual sodium carbonate was found nil in most of the area of Sohna block because combined concentration of carbonate and bicarbonate was less than combined concentration of calcium and magnesium. RSC varied between nil to 4.30 with average value of 0.32 meq L^{-1} in Sohna block. With increase in bicarbonates and carbonates concentration, increase in RSC value was observed by Amin (2014) and Yadav *et al.* (2016).

Impact of ground water on soil properties

The pH of soil profiles showed irregular trend with soil profile depth. It followed decreasing trend in some of the villages whereas in some of the villages, it increased with increase in soil profile depth. Reason behind highest value of pH in 0-15 cm soil depth was precipitation of calcium and magnesium carbonates in course of evaporation process due to presence of HCO_3^- ions along with exchangeable and soluble sodium. So, an indication of sodicity and salinity development was given by high value of pH of soil saturation extract. Vijaykant (2016) and Rathi *et al.* (2018) observed similar results. Due to increase in sodium and clay content, increase in pH with depth in soil profile was observed whereas decrease in pH value with soil depth might be due to release of hydrogen ion under influence of soluble cation present in applied irrigation water. Similar results were confirmed by Gandhi *et al.* (2009) and Tikkoo *et al.* (2010) and Jayaprakash *et al.* (2012). Maximum accumulation of salts was observed in surface layer of soil (0-15 cm) and with increase in depth of soil profile, EC of soil saturation extract showed decreasing trend. The process of evaporation, due to which soluble salts moved toward surface layer of soil by capillary action, might be the reason behind the high concentration of soluble salts in 0-15 cm depth of soil profile. As samples of soil profile were taken just before monsoon, the leaching of soluble salts might not taken place from surface to sub surface layer of soil profile. Decrease in calcium and magnesium concentration in soil saturation extract led to decrease in EC of soil saturation extract. Mediratta *et al.* (1985), Singh (2005), Qadir *et al.* (2007), Raghubanshi and Singh (2013), Vijaykant (2016) also confirmed similar results. High concentration of salts at lower depth in some of the villages were due to leaching of soluble salts might be the reason for increase in EC of soil saturation extract with depth. Results in line were observed by Singh (2005).

In the majority of the villages of the Sohna block, the saturation percentage of the soil profile had an erratic relationship with the depth of the soil profile. Similar findings were also reported by Arvind (2007). In several of the villages, such as Sanchauli in the Sohna block, the pore size shrank as the depth of the soil increased due to increased compaction. The same outcomes were noted by Mukesh (2003).

Calcium carbonate content was found nil at most of the soil sampling sites of Sohna block except some of the sites like Alipur, Sanchauli and Darbaripur. Dissolution and leaching of carbonate and bicarbonate in coarse textured soil and CO_2 evolution due to high temperature resulted in calcium carbonate content at these sites. Tikkoo *et al.* (2010) and Yadav *et al.* (2016) confirmed similar results.

Although concentration of all the cations and anions followed decreasing trend with increase in depth of soil profile but concentration of some of ions like Cl^-, SO_4^{2-}, Ca^{2+}, Na^+, Mg^{2+} increased at lower depth of 30-45 or 45-60 cm at some of the soil sampling sites. Maximum accumulation of cations and anions in surface layer of soil and decrease in their concentration with depth might be due to capillary action of water which results in movement of these ions toward surface layer of soil. Reason behind high concentration of ions in surface layer of soil profile might be sampling before monsoon and evaporation due to high temperature during summer months. The increase in EC at lower depth at some of the sites led to increase in concentration of some of the ions at lower depth of soil profile. More *et al.* (1988), Gandhi *et al.* (2009) and Vijaykant (2016) validated similar results

Conclusions

The findings showed that most of the Sohna block were under low-quality water, with the majority of that region being under saline water as compared to alkali water. So special management strategies based on climatic factors, soil texture and crops to be grown are essential for optimum utilization of poor-quality groundwater for sustainable crop productions without deteriorating the soil quality. Thus, conjunctive use of groundwater (blending of groundwater with canal water) is a good practice to reduce the harmful effect of groundwater and its utilization without deteriorating soil quality and without reducing crop yield.

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Effect of Micronutrients on Yield and Quality of Guava c*v* Lucknow 49 in Sodic Soil

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Abstract

Tamil Nadu occupies 4.7 lakh ha of salt-affected soils, in which 3.0 lakh ha area is under inland and 1.7 lakh ha is coastal. Among the 3.0 lakh inland salt affected soils, 2.0 lakh ha have developed alkalinity and 1.0 lakh ha salinity. In Trichirappalli, the study area a total 18,155 ha area falls under alkalinity problem. Guava is one of the commercial fruit crops comes well under salt-affected soils. The main problem in yield reduction in guava is due to micronutrient deficiency. Hence, the present investigation was conducted at Horticultural College and Research Institute for Women, Tiruchirappalli during 2020-2021 in clay loam texture with pH 9.23, ESP-36.52%. The experiment was laid out in Randomized Block Design (RBD) on guava cv. Lucknow -49 planted at 3m × 2m spacing with seven treatments replicated three and ten number of plants per replication were taken. The treatments comprised of T_1 – Control, T_2 – RDF alone (300:150:150 g NPK/tree), T_3 – RDF + 100% MN Mixture TN Govt. rec.@500g/tree,T_4 – RDF + 125% MN Mixture TN Govt. rec.@500g/tree ,T_5 – RDF + 100% Enriched FYM TN Govt. rec.@500g/tree,T_6 - RDF + 125% Enriched FYM TN Govt. rec.@500g/tree,T_7- RDF +1% foliar spray of 100% MN Mixture TN Govt (II approximation) rec .The foliar application of these treatments was made at three times of new flush, 30 and 70 days after flowering. The results revealed that the application of RDF + 100% Enriched FYM TN Govt. rec.@500g/tree recorded highest 50% flowering (33.6%), fruit retention (51.5%), highest yield (42.3 Mg ha^{-1}) and quality parameters like TSS (12.8^{0}brix) and lowest acidity (0.42%) followed by treatment RDF + 125% MN Mixture TN Govt. rec.@500g/tree and the same treatment recorded the BC ratio of 3.64 while the control with BC ratio of 2.75.

Key words: Salt-affected soils, Guava, Micronutrient mixture, Recommended dose of fertilizers (RDF)

Introduction

The area under guava in India is 235.6 thousand ha and production is 3198.3 thousand metric tonnes with a productivity of 13.6 metric tonnes per hectare (Horticulture Statistics at a Glance, 2018) Micronutrients play an important role in production and their deficiency leads in lowering the productivity (Singh, 2001).

Guava is very hardy, long lived and needs comparatively little attention. Guava is rich source of vitamin C, vitamin A, vitamin B12 (Riboflavin) and minerals like calcium, phosphate and iron. The vitamin C content of Guava fruit is 212 mg/ 100 g and pectin content (1.15%). Micro nutrient deficiency is a common physiological disorder seen in guava. Zinc deficiency results in interveinal chlorosis, formation of little leaves and restricted internodes leading to "rosette". This is common in water logged areas. This disorder can be rectified by spraying 500g of zinc sulphate + 350g of slaked lime dissolved in 72 litres of water. Two such sprayings have to be given at 15-30 days interval (Rawat *et al.,* 2010).

Guava has delightful taste and flavour. It is the fruit that has been often referred as "Apple of tropics." Bronzing in guava is a complex nutritional disorder When fruiting starts in a soil marginal in P and K, the nutrients are mobilised from older leaves to the fruits, causing bronze colour. Micronutrients like Fe, Zn, B, Cu, Mn, Mo and Cl play a vital role in plants. Micronutrients can be applied to plants by soil and foliar application. Foliar application of micronutrients is more successful than soil application.

Spraying of 0.3% boric acid 10-15 days before flowering corrects the deficiency. In general, foliar applications of 0.5 per cent zinc sulphate and 0.4 per cent boric acid 10 to 14 days before flowering effectively eliminate the zinc and boron deficiencies. Seeing the essential role of micronutrients in guava the present investigation was carried out to evaluate the micronutrient mixture on growth, yield and quality of guava.

Materials and Methods

The field trial was conducted at Horticultural College and Research Institute for Women, Tiruchirappalli during 2020-2021 in soil with clay loam texture, pH 9.23, and ESP 36.52%. The experiment was laid out in Randomized Block Design (RBD) with seven treatments replicated three times and ten number of guava plants per replication were planted at 3 m x 2 m spacing. The treatments comprised of T_1 – Control, T_2 – RDF alone (300:150:150 g NPK/tree),T_3 – RDF + 100% MN Mixture TN Govt. recommendation @500g/tree($FeSO_4$-80g,$MnSO_4$-50g,$ZnSO_4$-50g,$CuSO_4$-10g,Borax-125g,Sodium Molybdate - 2g)/tree ,T_4 – RDF + 125% MN Mixture TN Govt. rec.@500g/tree ,T_5 – RDF + 100% Enriched FYM (Mixing FYM and MN mixture at 10:1 and incubated for 30 days) TN Govt. MN mixture rec.@500g/tree,T_6 - RDF + 125% Enriched FYM TN Govt. rec.@500g/tree,T_7- RDF +1% foliar spray of 100% MN Mixture TN Govt (II approx) rec . The foliar application of these treatments was made at three times of new flush, 30 and 70 days after flowering. The initial soil samples were collected and analyzed for their physico-chemical properties. The growth parameters were recorded and the yield and quality parameters were analyzed and based on the performance economics was worked out.

Results and Discussion

Initial soil characteristics of the experimental field

The soil texture was clay loam with Alathur soil series. The initial soil pH was 9.23 and EC (1:2) 0.17 dS m^{-1} and ESP 36.5%. The available N was low (184 kg ha^{-1}), medium in available P (21.0 kg ha^{-1}) (Table 1). The available K was medium (232 kg ha^{-1}). The available micronutrients Fe was 3.52 ppm, deficit in Zn (0.71 ppm), the available Cu and Mn were 0.42 ppm and 0.39 ppm, respectively.

Table 1. Initial soil characteristics of experimental field

S. No.	Initial soil properties	Values	Rating
1	Soil texture	Clay loam – Alathur soil series	
2	ESP (%)	36.52	High
3	Organic carbon (g kg^{-1})	3.9	Low
4	Available Nitrogen (kg ha^{-1})	184	Low
5	Available Phosphorus (kg ha^{-1})	21.0	Medium
6	Available Potassium (kg ha^{-1})	232	Medium
7	Free $CaCO_3$ (%)	19.2	Calacareous
8	pH	9.23	Alkali
9	EC (dS m^{-1})	0.17	Low
10	Fe (ppm)	3.52	Low
11	Zn (ppm)	0.71	Low
12	Cu (ppm)	0.42	Low
13	Mn (ppm)	0.39	Low

Floral characters

The results indicated significant differences when applied micronutrient mixture on flower characters as represented in Table 2. As regards to the number of flowers, the treatment T_5 (RDF + 100% enriched FYM TN Govt. rec.@500g/tree) application recorded the maximum flowering % of 33.6% over the control which recorded 26.4%. The data on fruit retention revealed that the treatment T_5 registered highest fruit retention of 51.5 % followed by the treatment T_4(RDF + 125%

Table 2. Effect of micronutrients on flower characters in Guava cv Lucknow 49

Treatment	Particulars	Flowering (%)	Fruit retention (%)
T_1	Control	26.4	36.3
T_2	RDF alone (300:150:150 g NPK/tree)	27.5	44.1
T_3	RDF + 100% MN Mixture TN Govt. rec.@500g/tree	30.6	47.0
T_4	RDF + 125% MN Mixture TN Govt. rec.@500g/tree	30.9	48.3
T_5	RDF + 100% Enriched FYM TN Govt. rec.@500g/tree	33.6	51.5
T_6	RDF + 125% Enriched FYM TN Govt. rec.@500g/tree	32.3	35.5
T_7	RDF +1% foliar spray of 100% MN Mixture TN Govt (II approxmn.) rec	29.2	45.9
	SED	0.32	0.20
	LSD (p=0.05)	0.98	0.43

MN Mixture TN Govt. rec.@500g/tree) with a value of 48.3%. The treatment control recorded the lowest value of 36.3%. The production of a greater number of flowers per tree, fruit retention in this treatment could be due to zinc and iron which act as catalyst in the oxidation and reduction process and is also of great importance in the sugar metabolism thus increased the yield per tree. Increase in yield of guava fruits due to foliar application of micronutrients alone along with soil has been reported by several workers from different parts of country (Meena *et al.,* 2005; Trivedi *et al.,* 2012) which supported the results obtained in present investigation.

Yield and quality parameters

The yield and quality parameters were recorded as shown in Table 3. As regards to the fruit weight, T_5 application recorded the maximum weight of 174.3g followed by the treatment T_4 with a value of 170.2 g while the control T_1 recorded the value of 145.2 g per fruit

The fruit yield in terms of Mg ha^{-1} also revealed that the application of T_5 recorded the maximum yield of (42.3 Mg ha^{-1}) followed by T_4 41.8 Mg ha^{-1} while the control recorded the lowest value of 30.7 Mg ha^{-1} (Table 3). Increase in yield of guava fruits due to foliar application of micronutrients alone along with soil has been reported by several workers from different parts of country (Janaki *et al.,* 2020; Trivedi *et al.,* 2012)which supports the results obtained in present investigation.

Application of RDF along with Micronutrient mixture increased the nutrients availability and increase sucrose content of fruits. Application of RDF + 100% Enriched FYM TN Govt. rec.@500g/tree application recorded highest TSS^0 Brix value of 12.8^0 while the lowest was recorded in the control of 10.9^0 brix while the other treatments recorded the intermediate values. The acidity % was also influenced by different nutrient management practices. The treatments receiving RDF + 100% Enriched FYM TN Govt. rec.@500g/tree recorded the lowest acidity value of 0.42% followed by the next value of RDF + 125% MN Mixture TN Govt. rec.@500g/tree) of 0.48% while the lowest was recorded in control of 0.82%. These findings are in agreement with Rawat *et al. (*2010) and Zagade (2017) who reported that foliar application of zinc sulphate reduced the acid content in guava fruits.

Fruit nutrient analysis

The maximum Fe and Zn content in fruit was recorded in the treatment T_5 with values of 252 ppm and 17.2 ppm, respectively while minimum values of these parameters were noticed in the control T_1 (Farmers practice) with Fe (172 ppm) and Zn (7.50 ppm) while the other treatments showed the intermittent values (Table 4). The maximum Cu and Mn content in fruit was recorded in the treatment T_5 with values of 6.54ppm and 3.70 ppm, respectively while minimum values of these parameters were noticed in the control T_1 (Farmers practice) with Cu (1.80ppm) and Mn (1.40 ppm) while the other treatments shows the intermittent values. The increased in nonreducing sugar and total sugar with zinc sulphate alone or in combination with other micronutrients may be due to increased rate of photosynthesis and perceptible increase in

Table 3. Effect of micronutrients mixture on fruit weight, yield and quality characters in Guava under high density planting cv Lucknow 49

Treat-ment	Particulars	Yield (Mg ha^{-1})	Fruit weight (g)	Acidity (%)	TSS^0brix
T_1	Control	30.7	145.2	0.82	10.9
T_2	RDF alone (300:150:150 g NPK/tree)	36.6	155.1	0.76	11.3
T_3	RDF + 100% MN Mixture TN Govt. rec.@500g/tree	41.2	165.2	0.72	12.2
T_4	RDF + 125% MN Mixture TN Govt. rec.@500g/tree	41.8	170.2	0.48	12.4
T_5	RDF + 100% Enriched FYM TN Govt. rec.@500g/tree	42.3	174.3	0.42	12.8
T_6	RDF + 125% Enriched FYM TN Govt. rec.@500g/tree	40.1	160.3	0.58	12.3
T_7	RDF +1% foliar spray of 100% MN Mixture TN Govt(II approxmn.) rec	39.5	158.2	0.60	12.0
	SEd	0.84	0.32	0.10	0.08
	CD(5%)	1.72	0.70	0.23	0.12

Table 4. Effect of micronutrients mixture on Fe, Zn, Cu and Mn content in guava fruit

T.No.	Treatment Details	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
T_1	Control	172.00	7.50	1.80	1.40
T_2	RDF alone (300:150:150 g NPK/tree)	194.00	9.80	2.40	1.50
T_3	RDF + 100% MN Mixture TN Govt. rec.@500g/tree	258.00	15.60	4.20	2.85
T_4	RDF + 125% MN Mixture TN Govt. rec.@500g/tree	245.00	16.10	5.80	3.10
T_5	RDF + 100% Enriched FYM TN Govt. rec.@500g/tree	252.00	17.20	6.54	3.70
T_6	RDF + 125% Enriched FYM TN Govt. rec.@500g/tree	235.80	14.82	4.40	2.90
T_7	RDF +1% foliar spray of 100% MN Mixture TN Govt (II approxmn.) rec	203.00	11.50	2.27	2.10
	Mean	235.80	14.82	4.40	2.90
	SEd	2.01	0.16	0.08	0.03
	LSD (p=0.05)	4.39	0.22	0.11	0.05

Table 5. Cost economics of application of micronutrients

Treatment	Particulars	Yield (Mg ha^{-1})	B: C ratio
T_1	Control	30.7	2.75
T_2	RDF alone (300:150:150 g NPK/tree)	36.6	2.96
T_3	RDF + 100% MN Mixture TN Govt. rec.@500g/tree	41.2	3.45
T_4	RDF + 125% MN Mixture TN Govt. rec.@500g/tree	41.8	3.51
T_5	RDF + 100% Enriched FYM TN Govt. rec.@500g/tree	42.3	3.64
T_6	RDF + 125% Enriched FYM TN Govt. rec.@500g/tree	40.1	3.21
T_7	RDF +1% foliar spray of 100% MN Mixture TN Govt(II approxmn.) rec	39.5	3.01

sugar contents through the foliar feeding of zinc sulphate might be due to the active synthesis of tryptophan in the presence of zinc, the precursor of auxin which in turn causes an increase in rate of chlorophyll synthesis which ultimately accelerates the photosynthetic activity which accumulated more sugars in fruits. These findings are in agreement with Rawat *et al*. (2010).

Economics

Based on the yield obtained, cost economics was worked out. Based on the cost economics worked out, it was found that the Treatment T_5 recorded the highest BC ratio of 3.64 while the control with BC ratio of 2.75

Conclusions

Application of recommended dose of fertilizers (RDF- 300:150:150 g NPK/tree) + 100% Enriched FYM TN Govt. recommendation @500g/tree recorded highest (50%) flowering, and fruit retention (51.5%), fruit yield (42.3 Mg ha^{-1}) and quality parameters like TSS (12.8^{0}brix) and lowest acidity (0.42%) and the same treatment recorded the BC ratio of 3.64 which was followed by treatment RDF + 125% MN Mixture TN Govt. recommendation @500g/tree. It shows that micronutrients play very important role in guava cultivation.

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Physico-chemical Water Quality Parameters of Damanganga Estuary, Daman

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Abstract

The present study was carried out to study the physico-chemical water quality parameters of Damanganga estuary, Daman collecting and analyzing the samples from Damanganga estuary. The water quality parameters were observed and found to be as water temperature (27.83±0.72) ^{0}C, pH (8.35±0.05), salinity (10.83±2.39) part per thousand (ppt), dissolved oxygen (7.80±0.46) mg l^{-1}, chloride (5157.16±433.47) mg l^{-1}, total hardness (2633.33±147.57) mg l^{-1}, calcium hardness (1322.71±107.80) mg l^{-1}, magnesium hardness (1310.56±108.27) mg l^{-1} and total alkalinity (115±3.86) mg l^{-1}. Study revealed that chloride, total hardness, calcium hardness, and magnesium hardness were outside the range of WHO (1993), CPCB (2007), BIS (2012) and EPA (2009); and the water pollution index (WPI) was of category III (1.202) indicating that the Damanganga estuary was moderately polluted.

Key words: Estuary, Daman, Damanganga, Physicochemical parameters, Water quality, Water pollution index (WPI)

Introduction

Water is the most essential compound of the ecosystem, and its quality depends upon the physical, chemical, and biological characteristics of water. These characteristics are usually based on the utilization of water and its utility in various usages, such as domestic, agriculture, industrial, etc. These uses of water are designated based on water quality guidelines given by the World Health Organization (WHO), the Central Pollution Control Board (CPCB), the Bureau of Indian Standards (BIS), and the Environmental Protection Agency (EPA) for fresh water, coastal and marine water resources. These National and International standards compare with the analysis of water quality parameters; it is the statistical tools that decide whether or not to use the specific activates. The interaction of water quality with both physical and chemical properties plays a significant role in the composition, distribution, and abundance of aquatic communities. The characteristics of water bodies influence the quality of water individually and in combination with various pollutants, thereby influencing the biota in the aquatic web cycle. Many researchers and scientists have analyzed the quality of water based on the different methods of pollution indexing (Miliasevic *et al.,* 2011; Ujjania and Dubey, 2015). Singh *et al.* (2018) observed that the Sutjel River was polluted due to industrial discharge. Similarly, Semy and Singh (2019) found that the range of sulphate, free carbon dioxide (CO_2), biological oxygen demand (BOD), and pH were not within the permissible limits due to contamination of water catchments characterized by heavy metals discharged from coal mining effluents into the Tsurang River.

The variation in water quality parameters influences the natural activity and efficiency of marine organisms in estuary waters at Punnaikayal (Eucharista, 2019), while Malsawmtluanga, (2022) observed parameters of water samples from the different natural springs in Mualthuam 'N' and found that all the water sources are well within the permissible limits of BIS (2012) standards and could be consumed for domestic, agricultural, development, and other purposes. Global warming and sea level rise are also threatening problems in the current climate change scenario, which is also a reason for saline water intrusion into the coastal fresh water aquifers (Sajeena *el al.,* 2022). According to Nirmala *et al. (*2021) the indexing method of water quality in Theni and Dindigul Districts, Tamil Nadu, was analyzed using a weighed arithmetic index method to indicate critical conditions of water that affect the groundwater quality. It was found that the water quality ranged between excellent and unsuitable for drinking. Similarly, the Narmada River's comprehensive pollution index was analyzed and statistical tools were used. Statistical applications identified that pH; conductivity, alkalinity, and hardness are governing the water quality, whereas BOD and chloride have been influencing water quality in densely populated regions (Gupta *et al.,* 2020). The fluctuation of hydrobiological parameters in seasonality was assessed through advanced numerical tools and reflected the portability of water (Babita and Upadhyay, 2022); thus, quality of water and statistical tools represent the overall condition of water resources. With this background, the present study was carried out with the objective of assessing the water quality of the Damanganga estuary and the sustainability of aquatic life in it. The level of pollution is based on the indexing method, particularly the water pollution index (WQI), and it is major concern from the point of the stream and the flora and fauna supported by the estuary.

Materials and Methods

Study area

The present study was carried out at Damanganga estuary, Daman where the Damanganga River joins the Arabian Sea (Estuary), situated at 20°24′46″ N Latitude and 72°50′10″ E Longitude (Fig. 1) for the period of six months (December to May 2021).

Sample analysis

Surface water samples were collected on a monthly basis during the morning hours of 6:30 to 9:00 am from Damanganga estuary, Daman. Sampling was done at a monthly interval for a period of six months, from December 2020 to May 2021. These water samples were used to analyse the important water quality parameters, including water temperature, pH, salinity, dissolved oxygen (DO), chloride, total hardness, calcium hardness, magnesium hardness, and total alkalinity. Out of these parameters, water temperature, pH, and dissolved oxygen (DO) were analysed *in-situ*. For the remaining parameters, water samples were preserved and transported to the research laboratory. Water samples were preserved (acidification with sulphuric acid to pH < 2) in pre-rinsed plastic containers. The sampling containers were labelled and transported on the

Fig. 1 Map of Damanganga estuary, Daman

same day to the laboratory, Department of Zoology, Government College Daman. For the preservation and analysis of surface water samples within 72 hours, the standard methods of APHA (1995) were followed.

Statistical analysis

The water pollution index (WPI) represents the sum of the ratio between the observed parameters and regulated standard values used for the calculation of the WPI in Damanganga estuary to follow the equation (Lyulko *et al.,* 2001):

$$WPI = \Sigma (Ci/SFQS) \times (1/n)$$

Where Ci represents the average monthly concentration of the analysed water quality parameter, SFQS represents the standard values for the water quality standards of coastal water, CPCB, marine coastal water, etc., while n indicates the number of analysed parameters in the research. The statistical analysis of the data and graphic presentations were done in Microsoft Excel 2019.

Results and Discussion

The results of present investigation of water quality parameters and the status of WPI are depicted in Tables 1 and 2. The maintenance of a healthy aquatic ecosystem is dependent on the physico-chemical properties of water. Therefore, assessing the water quality is very important in determining the quality of the ecosystem and its pollution status. The water quality parameters of Damanganga estuary showed fluctuations due to freshwater flow of the river and tidal influx of the sea. Water temperature is important in relation to fish life as aquatic organisms show varied sensitivity. In the present study, the minimum (26.00) °C value of water temperature was observed in December 2020 and the maximum (31.00) °C in May 2021 with an average value (27.83±0.72) °C which was within the limit given by WHO (1993). Similar results were reported by Ujjania and Dubey (2015) in the Tapi Estuary. The water temperature fluctuated because of the intensity of solar radiation, evaporation, and freshwater mixing. Greater solar radiation and high atmospheric temperatures may be the reason in summer, while the lower observed during lower period may be attributed to the cold climate or weather.

Table 2. Classification of water pollution status on basis of water pollution index (WP)

Class	Characteristics	Degree of WPI
I	Very pure	<0.3
II	Pure	0.3-1.0
III	Moderately polluted	1.0-2.0
IV	Polluted	2.0-4.0
V	Impure	4.0-6.0
VI	Heavily impure	>6.0

(Lyulko *et al.,* 2001)

The pH is a measure of the acid-base balance of a solution, specifically the concentration of hydrogen ions (H^+). In aquatic ecosystems, pH is

Table 1. Water quality parameters in Damanganga estuary during the study period

Parameters	Unit	Minimum	Maximum	Average ± SE	Maximum permissible limits	Water pollution index (WPI)
Water temperature	°C	26.00	31.00	27.83±0.72	35[a]	0.133
pH	_	8.20	8.50	8.35±0.05	8.5[b]	0.164
Salinity	ppt	5.00	20.00	10.83±2.39	10-20[d]	0.090
D.O	mg l^{-1}	6.08	9.44	7.80±0.46	4.0[c]	0.325
Chloride	mg l^{-1}	3905.00	7080.00	5157.16±433.47	1000[b]	0.086
Total hardness	mg l^{-1}	2000.00	3000.00	2633.33±147.57	600[b]	0.073
Calcium hardness	mg l^{-1}	1000.79	1601.27	1322.71±107.80	200[b]	0.110
Magnesium hardness	mg l^{-1}	998.81	1599.21	1310.56±108.27	100[b]	0.218
Total alkalinity	mg l^{-1}	100.00	125.00	115±3.86	600[b]	0.003
WPI = Σ (Ci/SFQS) × (1/n)						1.202

a=WHO (1993), b= BIS (2012), c= CPCB (2007) d= EPA (2009)

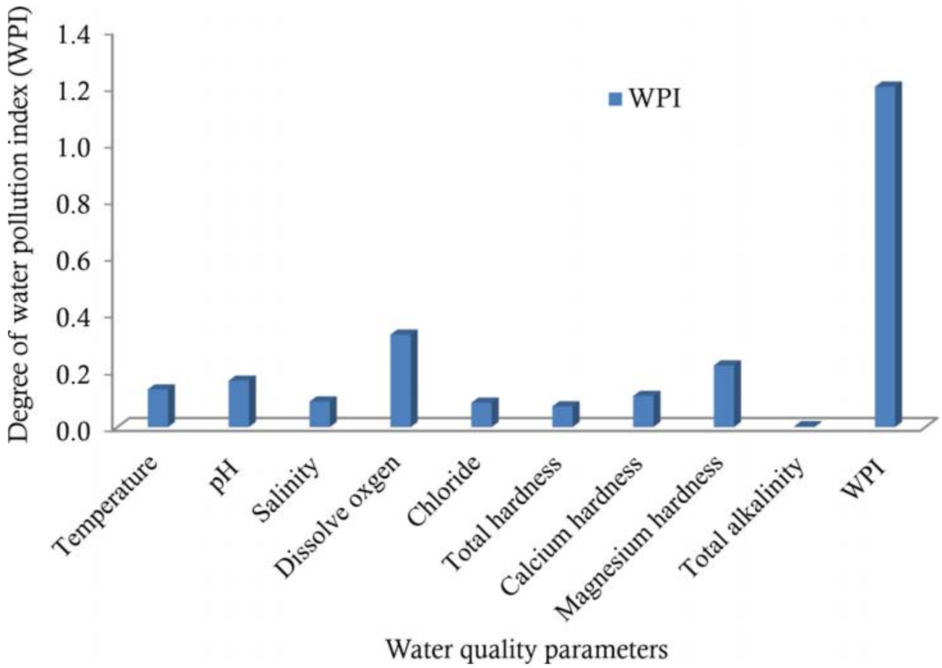

Fig. 2 Graphically representation of water pollution index (WPI)

important for biological productivity. Generally, pH values between 6.5 and 8.5 indicate good water quality. In a study, the pH of the ecosystem was lowest in January 2021 (8.20) and highest in April 2021 (8.50), with an average of 8.35±0.05. These values were within the acceptable range set by BIS (2012). Similar findings were reported by Pradhu *et al.* (2008). The high pH recorded may be due to increased algal growth and photosynthesis, as well as higher temperatures and microbial activity. The low pH may be influenced by freshwater influx and low photosynthetic activity. Salinity is the measurement of dissolved salts in water and it affects the types of plants and animals that can live in an aquatic ecosystem. In a monthly analysis, salinity levels fluctuated greatly, ranging from 5.00 to 20.00 ppt, with an average value of 10.83±2.39 ppt. This variation in salinity was caused by the influx of freshwater and marine water. A study conducted in Punnaikayal, Thoothukudi also found that higher salinity levels were influenced by factors such as higher solar radiation, the influx of marine water, and the decrease in adjacent marine water due to the flow of fresh water in coastal areas (Eucharista, 2019).

Dissolved Oxygen is an important factor for the health of aquatic ecosystems, as it is necessary for the survival of aquatic fauna. In a recent study, the minimum dissolved oxygen level was recorded in May 2021 (6.08) mg l^{-1}, while the maximum level was recorded in December 2020 (9.44) mg l^{-1}. The average dissolved oxygen level was 7.80±0.46 mg l^{-1}, which was within the limit set by the Central Pollution Control Board (CPCB) in 2007. Similar findings were observed in previous studies, where low dissolved oxygen levels were attributed to industrial organic effluents, sewage load, and high temperatures, while high dissolved oxygen levels were attributed to water aeration and low temperatures (Ujjania and Dubey, 2015).

In the present study it was found that chloride levels in water ranged from 3905 to 7080 mg l^{-1} from February to December, with an average value of 5157.16±433.47 mg l^{-1}. These levels exceeded the limit set by BIS (2012) indicating pollution. The high chloride concentration suggests the presence of marine water in freshwater, as well as pollution from sewage and industrial waste. This is consistent with a previous study on water quality in the Tapi estuary (Ujjania and Dubey, 2015).

Total hardness refers to the concentration of certain ions in water, such as calcium, magnesium, and alkaline earth metals. It is a measure of how water reacts with soap, with hard water requiring more soap to lather. In a recent study, the lowest and highest values of total hardness were found to be between (2000 and 3000) mg l^{-1} in the months of January 2021 and May 2021. The average value was 2633.33±147.57 mg l^{-1}, which exceeded the limit set by the Bureau of Indian Standards (BIS) in 2012. The high hardness levels were attributed to the tidal flow and the addition of sewage and detergents from residential areas (Ujjania and Dubey, 2015).

Calcium hardness is a common occurrence in water due to deposits of limestone and other calcium-bearing rocks. The hardness is caused by the salts of calcium and magnesium. In January 2021, the minimum calcium hardness value was 1000.79 mg l^{-1}, while in May 2021; the maximum value was 1601.27 mg l^{-1}. The average value was 1322.71±107.80 mg l^{-1}, which exceeded parameters of BIS (2012). This increase in calcium hardness can be attributed to tidal flow, decomposition of organic matter, and a higher proportion of calcium in the surrounding rocks and soil. Additionally, the addition of sewage water may also contribute to the increase in calcium hardness. A similar study conducted in Punnaikayal estuary, Tamil Nadu, by Eucharista, (2019) who reported similar findings. Magnesium hardness is a common element found in the earth's crust and natural waters. It contributes to the hardness of water along with calcium. In February 2021, the minimum value of magnesium hardness was 998.81 mg l^{-1}, while in March 2021; the maximum value was 1599.21 mg l^{-1}. The average value was 1310.56±108.27 mg l^{-1}, which exceeded BIS (2012) parameters. This suggests that the high concentration of magnesium hardness may be due to the influence of seawater and industrial effluent discharges in estuary water. Andrade *et al.,* 2011 also observed that the concentration of magnesium hardness exceeded the maximum permissible limit in Mangalore coastal water. Similarly, in Punnaikayal estuary, there was a trend of higher concentration during summer months and lower concentration during winter months (Eucharista, 2019).

Total alkalinity refers to the amount of dissolved compounds in water that can shift the pH towards neutrality or alkalinity. It is an important concept in determining a system's ability to buffer against acid impacts. The buffering capacity of a body of water refers to its ability to resist or dampen changes in pH. Alkaline compounds such as bicarbonate, carbonates, and hydroxides in water can remove H^+ ions and lower the acidity, thereby increasing the pH (UNEP, 2006). The change in alkalinity depends on the presence of carbonates and bicarbonates, which in turn depends on the release of CO_2. In a specific study, the minimum and maximum values (100 and 125) mg l^{-1} in January 2021 and December 2020, respectively, mean values (115±3.86) mg l^{-1} of total alkalinity in a river were within the limits set by BIS, 2012 indicating normal levels. However, higher values of total alkalinity were observed, possibly due to the presence of strongly alkaline industrial waste water, sewage, and organic waste releasing CO_2 in the estuary. These factors may have contributed to an increase in carbonate and bicarbonate levels, resulting in an increase in alkalinity (Gupta *et al.,* 2020).

The water pollution index (WPI) is a number that represents the level of pollution in a body of water based on various water quality parameters. In this study, the WPI for the Damanganga estuary was recorded as 1.202, falls under category III, indicating moderately polluted water (Lyulko *et al.,* 2001). This finding is similar to a study conducted in the Borska Reka River in Siberia (Miliasevic *et al.,* 2011).

Conclusions

The present work revealed that WPI (1.202) under the degree of water pollution was moderately polluted and water quality parameters (water temperature, pH, salinity, DO, and total alkalinity) were within the prescribed limits while other parameters (chloride, total hardness, calcium hardness, and magnesium hardness) were beyond the maximum permissible limits which clearly indicates that Damanganga estuary and river water is moderately polluted by industrial effluents, domestic sewage drains and other anthropogenic activities in Damanganga estuary. Need to followed appropriate management policy and rules & regulation to discharge effluents.

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Survey, Characterization and Mapping of Ground Water Quality and its Effect on Soil Properties in Gurugram Block of Haryana

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Abstract

In Gurugram block of Gurugram district, a survey was conducted to evaluate the quality of the groundwater and determine how it affected the soil qualities. Using a handheld GPS, 72 groundwater samples and 40 soil samples overall were taken at two sites, four depths each, in the Gurugram block of the district. The pH, EC, SAR and RSC of ground water ranged from 7.38 - 8.03, 0.75-5.58 (dS m^{-1}), 4.44 - 21.75 (mmol $l^{-1})^{1/2}$ and nil to 4.50 (me l^{-1}), respectively. The anion contents followed the pattern $Cl^{-}>SO_4^{2-}> HCO_3^{-}>NO_3^{-}>CO_3^{2-}$ whereas the cations were found to be in the following order: $Na^{+}> Mg^{2+}> Ca^{2+}> K^{+}$. The village Sehrawan had the lowest saturation rate (28.84%) and the village Kherki Majra had the highest saturation percentage (34.57%). The settlement of Gurugram Rural (1.22 dS m^{-1}) in the Gurugram block has the lowest EC of saturated extract. The Hamirpur village had the highest EC of saturated extract (6.12 dS m^{-1}) whereas Hamirpur (7.34) and Harsaru Garhi (8.43) were the villages with the lowest and highest pH values, respectively. The correlation coefficient revealed that electrical conductivity (EC) of soil saturation extract was significantly and positively correlated with EC (0.962), Cl^{-} (0.936) and Na^{+} (0.902) of groundwater used for irrigation.

Key words: Water quality, Electrical conductivity, Saturation percentage, RSC, SAR, Soil properties

Introduction

Quality of irrigation water is an important parameter and needs to be taken into consideration for crop production and soil health. In groundwater studies, appropriate and accurate laboratory analysis of water quality is very necessary for its effective utilization and suitability of water for human use such as drinking, industrial and agriculture is determined by hydro chemical studies. Water being an excellent solvent, it is imperative to have the knowledge of the geochemistry of dissolved constituents and way of reporting analytical data. Usually groundwater has base cations (Ca^{2+}, Mg^{2+}, Na^{+}), bicarbonate and pH in neutral to slightly alkaline range (Frengstad and Banks, 2000). Quality of water for irrigation is correlated to its impact on soils and crops as well as its management. The use of good quality irrigation water can help in production of crops of good quality with high yield (Islam and Shamsad, 2009). Agriculture needs to be sustainable, thus, soil and water management and monitoring the soil and water parameters are essential. In arid and semiarid locations, over-drafting and deteriorating ground water quality are seriously harming crop productivity (Boumans *et al.*, 1988).

Water quality in Haryana is degrading due to overuse of fertilizers and pesticides. Now a days due to various development activities and exploitation of water resources have increased to great extent due to which, quality as well as quantity of water available for use is affected severely. Because of pavement in urban areas, groundwater recharge is reducing and groundwater withdrawal is increasing affecting its availability (Kumar *et al.*, 2015). Limited work at block level on soil and water quality parameters has been studied so far. Therefore, an appraisal for the quality of irrigation water is essential for sound irrigation planning so as to assess any possibility of development of secondary salinization/sodification in Haryana. So, keeping in view the above facts, the present study was carried out.

Materials and Methods

Area and location

Gurugram district of Haryana located (Fig. 1) between 27°39′00″ North and 28°32′25″ North latitudes and between 76°39′30″ East and 77°20′45″ East longitudes (Maps of India, 2023). Gurugram Plain and Gurugram Undulating Plain with Aravalli Hills are the two main sub-parts of Gurugram District from a physiographic perspective.

Soil and climate

The majority of the Gurugram district's soils are classified as Ochrepts kinds, while Orthids-Fluvents and Ochrepts Ustrets-Ustalfs types are found in the district's central and south western regions, respectively. The Gurugram district has sandy to sandy-loam type of soil (DCO Haryana, 2011). The Gurugram district has sub-tropical continental monsoon climate. The highest daily mean temperature of 40.2° C occurs in month of May. The average rainfall of Gurugram district during year 2018 was 386.7 mm (IMD, 2018).

Collection of water samples

To evaluate the quality of the groundwater, 72 water samples were taken from tube wells in the Gurugram block during 2018–19. A hand-held GPS is used to record accurate sampling site (longitude and latitude).

Analysis of water samples

After collection of water samples, filtration was carried out in laboratory and one or two drops of toluene were added to prevent microbial growth. Then ground water samples were analysed for pH and EC using pH meter and conductivity meter. Among soluble cations, Calcium and Magnesium were determined using Versante titration method (Diehl *et al.*, 1950), sodium and potassium using flame photometer and the anions (CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-) by their standard analytical procedures. Residual sodium carbonate (RSC) and sodium adsorption ratio (SAR) were worked out by using the formula given by Richards (1954) and Raghunath (1987), respectively.

Characterization of irrigation water

Sodium adsorption ratio (SAR) and residual sodium carbonate (RSC) were calculated by using the following equations, respectively for the

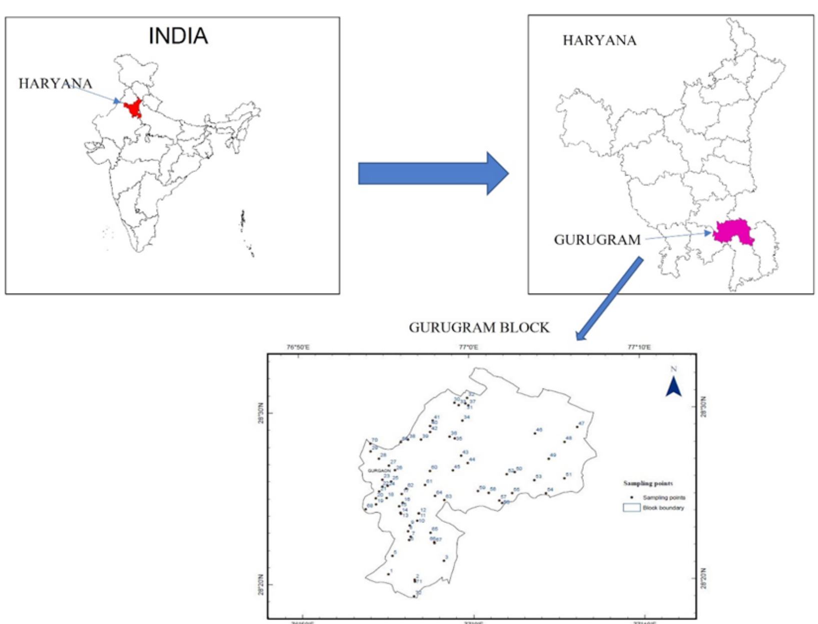

Fig. 1 Location Map of the study area

Table 1. Irrigation water quality classification criteria (AICRP on Management of Soil Affected Soils and Use of Saline Water in Agriculture, 1989*)

Sr. No.	Quality of water	Class	Quality parameters		
			EC (dS m^{-1})	SAR (mmol $l^{-1})^{1/2}$	RSC (mel^{-1})
1	Good	A	<2	<10	<2.5
2	Saline	B	-	-	-
	Marginally saline	B_1	2-4	<10	<2.5
	Saline	B_2	>4	<10	<2.5
	High SAR saline	B_3	>4	>10	<2.5
3	Alkali water	C	-	-	-
	Marginally alkali	C_1	<2	<10	2.5-4
	Alkali	C_2	<2	<10	>4
	Highly alkali	C_3	Variable	>10	>4

*Tiwari and Sharma (1989)

purpose of classification of water quality:

$$SAR\ (mmol\ l^{-1})^{1/2} = Na^+ / [(Ca^{2+} + Mg^{2+})/2]^{0.5}$$

$$RSC\ (me/l) = [CO_3^{-2} + HCO_3^-] - [Ca^{+2} + Mg^{+2}]$$

Water quality has been characterized as per AICRP (1989) has been shown in table 1.

Determination of soil properties

Soil samples were collected from 0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm depth of fields with the help of auger depending upon the results of ground water quality for fulfilling the purpose of determination of impact of groundwater quality on various soil properties. Soil samples were collected from same field from where groundwater samples of different categories were collected. A total of 10 sites were selected, 2 from each category of water quality for sampling of soil samples in Gurugram block on the basis of results of water quality analysis. Soil samples were analyzed for EC (1:2), pH (1:2), $CaCO_3$, saturation percentage as per standard procedures. For preparation of soil saturation paste, 2 mm sieved soil sample was taken in a beaker and distilled water added to it while stirring with spatula. This method of preparation of soil saturation paste was explained by US Salinity Laboratory Staff, 1954. Then saturation extract collected from soil saturation paste was used for analysis of various soil properties such as, EC (1:2), soluble cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and anions (CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}) as per standard procedures as described for water analysis (Richards, 1954).

Statistical methods

Karl Pearson method of correlation was used for computation of correlation coefficient among various parameters of groundwater and soil extracts (Panse and Sukhatme, 1954).

Results

Quality of irrigation water

The values of different water quality parameters were depicted in Table 2. The result of analysis of ground water samples of Gurugram block showed that pH, EC, SAR and RSC ranged from 7.38 - 8.03, 0.75-5.58 (dS m^{-1}), 4.44 - 21.75 (mmol l^{-1})1/2 and nil to 4.50 (me l^{-1}), respectively. The lowest value of pH (7.38), EC (0.75 dS m^{-1}) and SAR [4.44 (mmol l^{-1})1/2] found in village Hamirpur, Sehrawan and Nawada Fatehpur, respectively while the highest value of pH (8.03), EC (5.58 dS m^{-1}) and SAR [21.75 (mmol l^{-1})1/2] found in village Nawada Fatehpur, Hamirpur and Hamirpur, respectively. The cations were found to be in the order of $Na^+ > Mg^{2+} > Ca^{2+} > K^+$ and ranged from 5.58 to 47.40 meq l^{-1}, 1.10 to 15.75 meq l^{-1}, 0.40 to 5.75 meq l^{-1}, 0.19 to 0.66 meq l^{-1} respectively while anions followed in order of $Cl^- > SO_4^{2-} > HCO_3^- > NO_3^- > CO_3^{2-}$ and varied from 2.80 to 44.40 meq l^{-1}, 1.20 to 13.90 meq l^{-1}, 0.70 to 6.00 meq l^{-1}, nil to 0.80 meq l^{-1}, nil to 1.60 meq l^{-1} respectively. The spatial variability of EC, pH, RSC and SAR is represented by fig. 4, 5, 6 and 7 respectively.

Table 2. Chemical composition of tubewell water used for irrigation in different villages of Gurugram block

Properties	Villages of Gurugram block									
	Sehrawan	Nawada Fatehpur	Dhani Fazilpur	Kherki Majra	Badha	Naharpur Kasan	Hamirpur	Sadhrana Bamripur	Wazirpur	Garhi Harsaru
EC	0.75	1.72	2.58	2.23	5.26	4.17	5.58	1.58	2.21	2.81
pH	7.89	8.03	7.75	7.77	7.78	7.81	7.38	7.63	7.63	7.59
CO_3^{-2}	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1.60
HCO_3-	1.00	3.00	0.70	2.50	6.00	1.50	4.25	6.00	7.50	5.30
Cl^-	2.80	10.0	18.90	15.30	32.00	24.00	44.40	6.40	9.90	16.10
SO_4^{-2}	1.20	2.70	4.40	3.80	10.30	13.90	7.55	1.60	3.10	3.50
NO_3^-	0	0.80	0.22	0.40	0.72	0.39	0.58	0.05	0.52	0.45
Ca^{+2}	0.40	1.50	1.80	1.50	5.75	3.00	2.40	0.90	0.80	0.70
Mg^{+2}	1.10	5.50	5.90	4.70	15.75	9.00	7.10	2.50	2.20	2.10
Na^+	5.58	8.30	17.10	15.23	26.74	28.70	47.40	9.57	18.90	24.75
K^+	0.66	0.36	0.20	0.63	0.44	0.28	0.57	0.22	0.19	0.27
RSC	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2.60	4.50	4.10
SAR	6.44	4.44	8.71	8.65	8.16	11.72	21.75	7.34	15.43	20.92
Category AICRP	A	A	B_1	B_1	B_2	B_3	B_3	C_1	C_3	C_3

AICRP (1989) classification of groundwater quality of Gurugram block

The water samples were classified based on the criteria given by All India Coordinated Research Project (AICRP) on "Management of Salt Affected Soils and Use of Saline Water in Agriculture" (1989) in which water quality has been grouped into three categories such as good, saline and alkali. This classification is based upon EC, SAR and RSC Parameters. Based on the limits of various parameters, two poor quality water classes have been further classified each into 3 subclasses. The data pertaining to classification of groundwater quality as per AICRP (1989) criteria according to which 20 groundwater samples were recorded under good (A) category, 27 under marginally saline (B_1) category, 2 under saline (B_2) category,19 under high SAR saline (B_3) category, 2 under marginally alkali (C_1) category and 2 under high alkali (C_3) category is presented in table 5. Fig. 2 and 3 represents the distribution percentage of water samples and spatial variability of groundwater quality in different water quality categories of Gurugram block. According to AICRP (1989) criteria of categorization of groundwater quality, the quality of water of village Sehrawan and Nawada Fatehpur (Good quality), Dhani Fazilpur and Kherki Majra (Marginally saline), Badha (Saline), Naharpur Kasan and

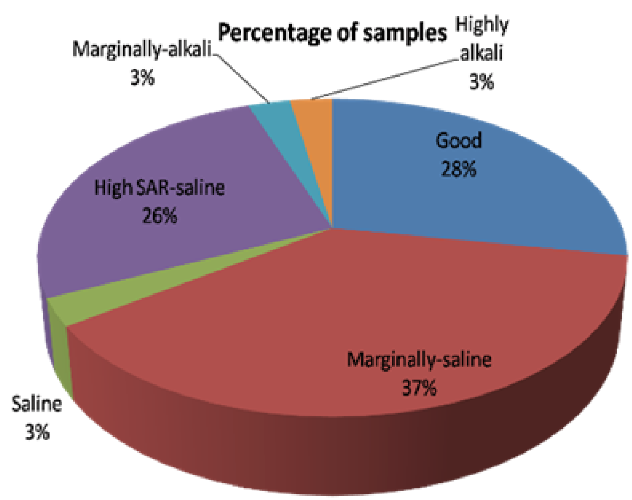

Fig. 2 Water quality distribution as per AICRP (1989**)**

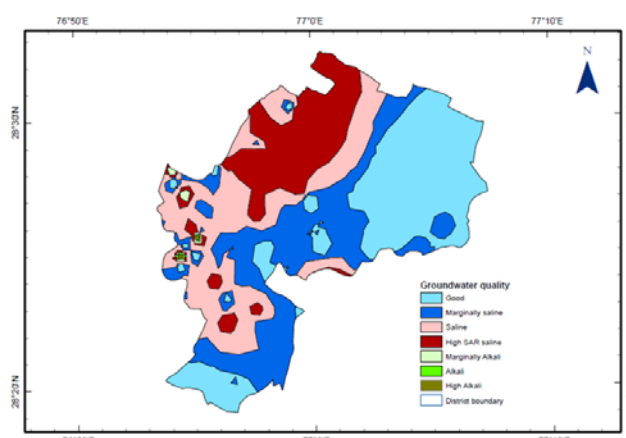

Fig. 3 Spatial variability of groundwater quality of Gurugram block

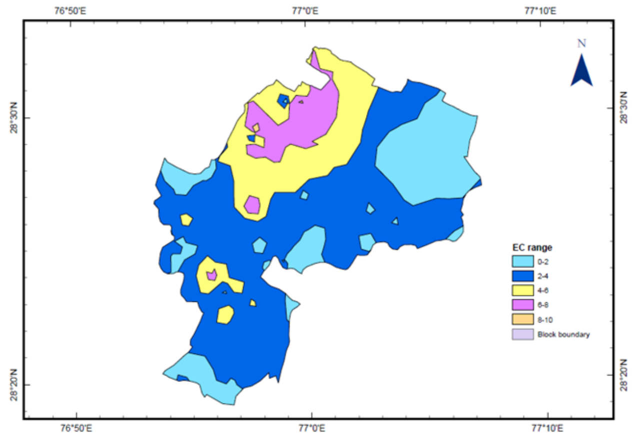

Fig. 4 Spatial variability in EC of groundwater of Gurugram block

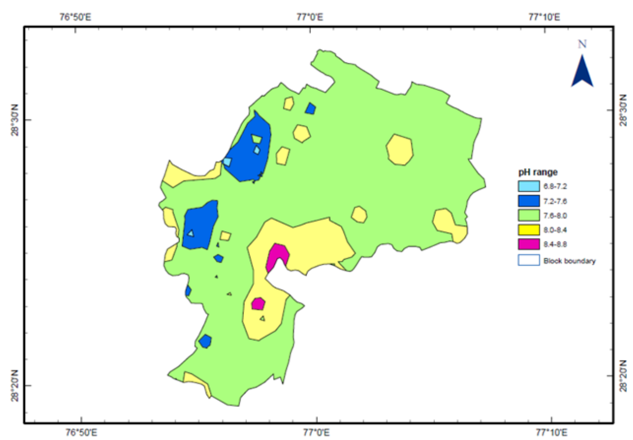

Fig. 5 Spatial variability in pH of groundwater of Gurugram block

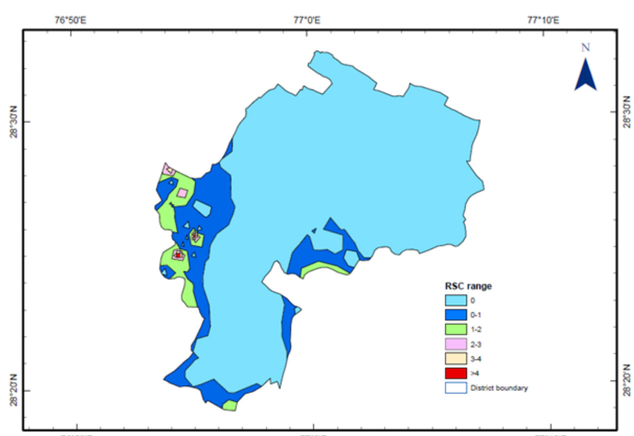

Fig. 6 Spatial variability in RSC of groundwater of Gurugram block

Hamirpur (High SAR value water), Sadhrana Bamripur (Marginally alkali), Wazirpur and Garhi Harsaru (High alkali water) were found.

Chemical properties of soil

The data related to chemical properties of soil

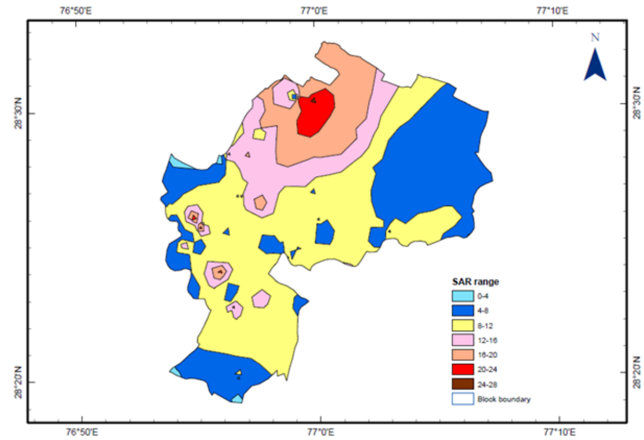

Fig. 7 Spatial variability in SAR of groundwater of Gurugram block

were illustrated in Table 3. In the Gurugram block village of Sehrawan (0.93 dS m^{-1}), where water of good quality (A) category was used for irrigation, the mean lowest EC of saturation extract was found. The village of Hamirpur (6.12 dS m^{-1}) in Gurugram block, where high SAR saline (B3) category water was used for irrigation, had the mean highest EC of saturation extract. Village Hamirpur and GarhiHarsaru had the mean lowest and mean highest pH readings, respectively, of 7.34 and 8.43. The village of Sehrawan had the lowest saturation rate (28.84%) and the settlement of Kherki Majra had the highest saturation percentage (34.57%). With the exception of the villages of Nawada Fatehpur and Dhani Fazilpur, none of the Gurugram soil sampling locations contained any calcium carbonate. Average accumulation of cations and anions was found to be in the following order: $Na^{+}>Ca^{2+}>Mg^{2+}>K^{+}$ and $Cl^{-}> HCO_3^{-}> SO_4^{2-}>CO_3^{2-}$ respectively.

Statistical analysis of different water quality parameters and soil saturation extract of Gurugram block

The correlation coefficient revealed that electrical conductivity (EC) of soil saturation extract was significantly and positively correlated with EC (0.962), Cl^{-} (0.936) and Na^{+} (0.902) of groundwater used for irrigation. Similarly, Cl^{-} and Na^{+} of soil saturation extract were significantly and positively correlated with EC (0.926), Cl^{-} (0.895) and EC (0.888), Na^{+} (0.966) of groundwater used for irrigation, respectively. The

Table 3. Effect of ground water quality on soil properties of different villages of Gurugram block

Properties	Villages of Gurugram block									
	Sehrawan	Nawada Fatehpur	Dhani Fazilpur	Kherki Majra	Badha	Naharpur Kasan	Hamirpur	Sadhrana Bamripur	Wazirpur	Garhi Harsaru
EC	0.93	2.27	3.59	3.79	5.57	5.42	6.12	2.00	3.13	3.59
pH	7.81	7.93	8.21	7.94	7.94	8.11	7.34	7.73	8.17	8.43
CO_3^{-2}	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1.78
HCO_3-	0.89	7.40	9.91	11.13	12.39	15.66	21.61	7.93	13.89	16.36
Cl^-	5.80	11.61	20.58	21.88	36.46	26.70	30.06	9.91	14.60	15.03
SO_4^{-2}	2.05	2.94	4.43	4.31	6.16	11.14	8.81	1.04	2.65	2.10
Ca^{+2}	3.66	4.24	8.63	11.29	15.30	12.24	12.20	5.18	2.30	2.61
Mg^{+2}	1.03	6.00	7.26	7.24	13.88	9.09	7.30	1.69	4.93	5.16
Na^+	4.21	11.65	18.96	17.38	24.72	30.94	39.03	11.69	22.83	26.24
K^+	0.20	0.76	0.75	1.05	1.31	1.56	2.13	0.72	0.95	1.26
Saturation (%)	28.84	29.70	30.97	34.57	30.80	29.97	31.43	32.43	30.61	30.81
$CaCO_3$ (%)	Nil	0.52	0.70	Nil	Nil	Nil	Nil	Nil	Nil	Nil

data pertaining to correlation coefficient between groundwater and soil parameters of Gurugram block is presented in Table 4.

Discussion

Quality of ground water

The electrical conductivity (EC) of groundwater samples collected from Gurugram block varied between 0.69 to 8.95 dS m^{-1} with average EC of 3.32 dS m^{-1} and the pH value of groundwater samples varied between 6.88 to 8.85 with average value of 7.83. According to the pH value, water of Gurugram block can be classified as slightly acidic to alkaline in nature. Higher value of pH could be due to high concentration of ions such as Sodium and bicarbonates. Bicarbonates produce hydroxyl ion which is reason for increase or decrease in pH value. Mukesh (2003), Deshmukh (2012), Kumar (2015), Mandal *et al.* (2016), Pal (2017) obtained similar results. Sodium was recorded as governing cation among all the cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) examined. High concentration of sodium resulted due to sodium compounds' water solubility and nature to be present in aqueous solution and cation exchange of groundwater mineral. Surface soil's salt leaching due to irrigation water or rain water could also be reason behind the dominance of Na^+ and Cl^- in the groundwater. It was found that concentration of all cations analyzed increased with increase in value of EC in the block except potassium which followed a steady trend. It was also observed that degree of increase in sodium and magnesium concentration was much higher than other anions. Results of the study were in

Table 4. Correlation coefficient between ground water and soil parameters of Gurugram block

	EC_{iw}	EC (soil)	pH (water)	pH (soil)	Cl^- (water)	Cl^- (soil)	Na^+ (water)	Na^+ (soil)
EC_{iW}	1	-	-	-	-	-	-	-
EC (soil)	.962**	1	-	-	-	-	-	-
pH (water)	-.498	-.462	1	-	-	-	-	-
pH (soil)	-.286	-.139	.280	1	-	-	-	-
Cl^- (water)	.974**	.936**	-.528	-.377	1	-	-	-
Cl^- (soil)	.926**	.949**	-.298	-.161	.895**	1	-	-
Na^+(water)	.926**	.902**	-.708*	-.285	.933**	.766**	1	-
Na^+ (Soil)	.888**	.918**	-.668*	-.087	.868**	.757*	.966**	1

**Correlation is significant at p= 0.01 level of significance

*Correlation is significant at p= 0.05 level of significance

Table 5. AICRP (1989) classification of groundwater quality of Gurugram block

Water quality	Class	Number of samples	Percentage
Good	A	20	27.78
Saline waters	B	-	-
Marginally-saline	B_1	27	37.50
Saline	B_2	2	2.78
High SAR-saline	B_3	19	26.38
Alkali waters	C	-	-
Marginally-alkali	C_1	2	2.78
Alkali	C_2	0	0.00
Highly alkali	C_3	2	2.78
		72	100.00

line with Shahid *et al.* (2008), and Rathi *et al.* (2018). Ion exchange of minerals with soils and rocks surrounding the groundwater might be the reason behind the presence of magnesium in groundwater whereas concentration of potassium and carbonates was very low and negligible, respectively

In case of anions, chloride was recorded as governing anion among all the anions (CO_3^{2-}, HCO_3^-, Cl, SO_4^{2-}, NO_3^-) analyzed. After that order of dominance of anions was sulphate, bicarbonate, nitrate and carbonate in groundwater of the block. It was observed that HCO_3^-, Cl^-, SO_4^{2-} and NO_3^- concentration have positive and significant correlation with EC of groundwater samples. In all the EC classes, sodium and chloride were the major cation and anion, respectively. Sharma, (1998), Rajpaul *et al.* (2014), Sanjay *et al.* (2016) and Ramprakash *et al.* (2020) obtained similar results. In contrast to bicarbonates concentration, concentration of carbonates was very low in groundwater. Dissolution of carbonic acid of aquifers and weathering of carbonates can be the reason for presence of CO_3^{2-} and HCO_3^- ions in water samples. Oxidation of pyrite, sulphur in igneous rocks, merasite, sulpharite and solution of other sulphur bearing minerals results in presence of sulphate ion concentration in groundwater. Findings are in agreement of results recorded by Pradhan *et al.* (2011) and Rahman *et al.* (2013). Industrialization, urbanization and fertilizers and chemicals application led to occurrence of nitrate concentration in groundwater of Gurugram block of Gurugram district. Shahid (2004), Jitender (2006) and Kumar and Kumar (2015) also found nitrate concentration in ground waters of Julana block of Haryana, Karnal block of Karnal, Haryana and Kisanganj district of Bihar, Rewari/Bawal block of Hrayana, Kaithal district of Haryana and Firozpur-Jhirka and Punhana block of Haryana, respectively.

The SAR value of groundwater sample taken from Gurugram block of Gurugram district varied between 1.41 to 25.26 meq $L^{-1}$with average value of 10.56 $(mmol^{-1})^{1/2}$. Combination of Sodium with carbonates and bicarbonates results in sodicity hazard due to removal of exchangeable calcium and magnesium from soil solution. Sodic condition due to decrease in exchangeable calcium and calcium precipitation results from usage of water with high SAR value. With increase in concentration of sodium ion, increase in SAR value of groundwater samples was also observed by Singh *et al.* (2011) and Kumar (2015). Residual sodium carbonate was found nil in most of the area of Gurugram block because combined concentration of carbonate and bicarbonate was less than combined concentration of calcium and magnesium. RSC varied between nil to 4.30 with average value of 0.32 meq L^{-1} in Gurugram block. With increase in bicarbonates and carbonates concentration, increase in RSC value was observed by Amin (2014) and Yadav *et al.* (2016).

Impact of ground water on soil properties

The pH of soil profiles showed irregular trend with soil profile depth. It followed decreasing trend in some of the villages whereas in some of the villages, it increased with increase in soil profile depth. Reason behind highest value of pH in 0-15 cm soil depth was precipitation of calcium and magnesium carbonates in course of evaporation process due to presence of HCO_3^- ions along with exchangeable and soluble sodium. So, an indication of sodicity and salinity development was given by high value of pH of soil saturation extract. Vijaykant (2016) and Rathi *et al.* (2018) observed similar results. Due to increase in sodium and clay content, increase in pH with depth in soil profile was observed whereas decrease in pH value with soil depth might be due to release of hydrogen ion under influence of soluble cation present in applied irrigation water. Similar results were confirmed by Gandhi *et al.* (2009) and Tikkoo *et al.* (2010) and Jayaprakash *et al.* (2012). Maximum accumulation of salts was observed in surface layer of soil (0-15 cm) and with increase in depth of soil profile, EC of soil saturation extract showed decreasing trend except in some villages like Nawada Fatehpur and Dhani Fazilpur, EC of saturation extract decreased up to 15-30 or 30-45 cm depth and then increased in 30-45 or 45-60 cm depth. High concentration of salts at lower depth due to leaching of soluble salts might be the reason for increase in EC of soil saturation extract with depth. Results in line were observed by Singh (2005). Decrease in calcium and magnesium concentration in soil saturation extract led to decrease in EC of soil saturation extract. Mediratta *et al.* (1985), Singh (2005), Qadir *et al.* (2007), Raghubanshi and Singh (2013), Vijaykant (2016) also confirmed similar results.

In the majority of the villages in the Gurugram block, the saturation percentage of the soil profile had a strange relationship to the depth of the soil profile. Similar findings were also reported by Arvind (2007). In several of the villages, such as Hamirpur in the Gurugram block, the pore size shrank as the depth of the soil increased due to increased compaction. The same outcomes were noted by Mukesh (2003). Calcium carbonate content where found was due to dissolution and leaching of carbonate and bicarbonate in coarse textured soil and CO_2 evolution due to high temperature resulted in calcium carbonate content at these sites. Tikkoo *et al.* (2010) and Yadav *et al.* (2016) confirmed similar results.

Although concentration of all the cations and anions followed decreasing trend with increase in depth of soil profile but concentration of some of ions like Cl^-, SO_4^{2-}, Ca^{2+}, Na^+, Mg^{2+} increased at lower depth of 30-45 or 45-60 cm at some of the soil sampling sites. Maximum accumulation of cations and anions in surface layer of soil and decrease in their concentration with depth might be due to capillary action of water which results in movement of these ions toward surface layer of soil. Reason behind high concentration of ions in surface layer of soil profile might be sampling before monsoon and evaporation due to high temperature during summer months. The increase in EC at lower depth at some of the sites led to increase in concentration of some of the ions at lower depth of soil profile. More *et al.* (1988), Gandhi *et al.* (2009) and Vijaykant (2016) validated similar results

Conclusions

The results revealed that most of the areas of Gurugram block were under poor-quality water with maximum area under saline water in contrast to alkali water. So special management strategies based on climatic factors, soil texture and crops to be grown, are essential for optimum utilization of poor-quality groundwater for sustainable crop productions without deteriorating the soil quality.

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STCR – IPNS Technology- A Boon for Sodic Soil Productivity Under Rice

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Abstract

The field experiment was conducted in Anbil Dharmalingam Agricultural College & Research Institute, Tiruchirapalli with rice along with set of Soil Test Crop Response (STCR) – Integrated Plant Nutrition System (IPNS) treatments. The soil of the experimental field was fine mixed iso-hyperthemic, sodic Vertic Ustropept, Alathur series with sandy clay loam texture. The experimental soil was sodic, characterized by high pH 9.02, EC 1.15 dS m^{-1} and ESP 51.23%. The field was reclaimed with required quantity of gypsum based on gypsum requirement (GR) calculation, along with continuous ponding of the field with rainwater followed by draining. Fertilizer prescription equations (FPEs) for rice on sodic soil have been developed and ready reckoner of fertilizer doses was computed for desired yield target (6 Mg ha^{-1}) of rice. The basis for making the fertilizer prescriptions viz. nutrient requirement (NR), contribution of nutrients from soil (Cs), fertilizer (Cf) and farmyard manure (Cfym) were computed using the field experimental data. The quantity of fertilizers contributed by the application of FYM was assessed. Application of FYM @ 12.5 Mg ha^{-1} with a manurial composition of N: 0.60%, P: 0.30%, K: 0.50%; and moisture: 30% contributed 42:18:32 kg ha^{-1} of fertilizer N, P_2O_5 and K_2O from the recommended dose of fertilizers based on soil test values. The deviation recorded in the achievement of targets aimed at was within the range of ± 10% (90 – 110%) proving the validity of the developed FPEs. Thus, this approach provides a strong basis for soil fertility maintenance consistent with efficient nutrient management and maintenance of soil productivity in sodic soils for sustainable and enduring agriculture.

Key words: Response ratio, Soil efficiency, Soil test, Crop Response, Sodicity

Introduction

Nearly 7.0 million ha (Mha) of agricultural land is affected by varying degrees of salt problems in the country (Sparks, 2003). The affected area is likely to increase in the near future due to secondary salinization in irrigation commands and lift irrigated schemes, increase in dependence of agriculture on poor quality waters in semi-arid and arid regions, sea water intrusion and brackish water aquaculture in coastal regions, by 2025, the area projected under salt affected soils in India is about 13 Mha. In Tiruchirappalli district, out of the total geographical area of 4,40,412 ha, 7,362 ha are slightly salt-affected (pH 8.2 to 9.0), 10,729 ha are moderately salt- affected (pH 9.0 to 9.6) and 64 ha are strongly salt- affected (pH >10) (Sharma *et al.,* 2021).

Alkali or sodic soil is defined as a soil having a conductivity of saturation extract less than 4 dS m^{-1} and an exchangeable sodium percentage greater than 15. The pH is usually between 8.5 – 10.0. Most alkali soils, particularly in the arid and semi-arid regions, contain $CaCO_3$ in the profile in some form and constant hydrolysis of $CaCO_3$ sustains the release of OH ions in soil solution. The OH ions released result in the maintenance of higher pH in calcareous alkali soils than that in non – calcareous alkali soils. Sodic soils are inherently low in organic matter (<0.1%), and available N, and are more responsive to N application. These soils are more prone to N losses due to higher N volatilization caused by high pH, further aggravating N deficiency. Microbial activity, which influences N mineralization, is restricted by salt stress (Ghosh *et al.*, 2014).

Therefore, the requirement of added N in sodic soils is higher than in normal soils and salt tolerant varieties appear to respond better to higher N application than sensitive varieties. Apparently, the lack of high yielding salt tolerant rice varieties and good management strategies specific for sodic soils are the main reasons for low and unstable productivity.

Grain yield of rice in salt-affected soils is much lower because of its high sensitivity to salt stress (Sherene *et al.*, 2019). Rice is exceptionally sensitive to salinity and sodicity at early seedling stage and high yield losses have been observed because of high mortality and poor crop establishment. Modern high yielding varieties require considerable investment to ameliorate these soils to ensure reasonable yields, but this investment is beyond the capabilities of the resource-limited smallholder farmers living in these salt affected areas. Chaubey *et al.* (2015) observed that the number of seedlings per hill and plant spacing were important factors determining plant population per unit area for optimum nutrient uptake and for accessing sufficient light for photosynthesis, which ultimately determine grain yield. Plant mortality in sodic soils is high when young seedlings are transplanted, adversely affecting plant establishment and growth.

Among the various methods of formulating fertilizer recommendations, the one based on yield targeting is unique in the sense that this method not only indicates soil test-based fertilizer dose but also the level of yield the farmer can hope to achieve, if good cultivation package is followed (Velayutham, *et al.*, 2016). In the "Inductive Approach" of Soil Test Crop Response (STCR) field experimentation, all the needed variation in soil fertility level is obtained not by selecting soils at different locations as in earlier agronomic trials, but by deliberately creating it in one and the same field experiment to reduce heterogeneity in the soil population (types and units) studied, management practices adopted and climatic conditions.

Ramamoorthy and Velayutham (2011) have elaborated this Inductive approach and the STCR field design, which is also quoted by Black (1993). The experimental data can be used for developing fertilizer recommendations for maximum yield and profit and for desired yield targets of crops. The fertilizer prescription equations developed using this model can be applied to sodic Vertisols of all tropical regions by substituting the soil nutrient status of the field for rice crop. Because of the above facts, the present investigation was contemplated in rice on Vertisol under sodic soil so as to elucidate the significant relationship between soil test values and crop response to fertilizers, to refine fertilizer prescription equations under IPNS for desired yield target of rice under sodic soil.

Materials and Methods

The field experiment was conducted in typical sodic soils of Agriculture College farm, Tiruchirapalli, TNAU, Tamil Nadu, Southern India. The farm is located in the Cauvery Delta Zone of Tamil Nadu at 10°15′ and 11°2′ N latitude and 78°10′ to 79°5′ E longitude at an altitude of 90 m above MSL. The experiment was conducted during October 2019 to March 2020. The soil of the experimental field belongs to Allathur soil series taxonomically referred as Vertic Ustropept, hyper thermic family of sodic Natustalf exhibiting sandy loam texture, alkaline reaction (pH 9.02) and non–saline conditions (EC 1.15 dS m^{-1}) and exchangeable sodium percentage (ESP) of 51.23. The field was reclaimed with required quantity of gypsum based on Gypsum Requirement (GR) calculation (1.0 Mg ha^{-1}) along with continuous ponding of the field with rain water followed by draining. This procedure was repeated three times till the required ESP reduction i.e., below 15 and the final ESP was 15.53. The initial soil fertility status showed low organic carbon (4.1 g kg^{-1}), low available N (224 kg ha^{-1}), high available P (22.7 kg ha^{-1}), medium available K (250 kg ha^{-1}). The available Zn, Cu, Fe and Mn were in the sufficient range (7.5, 5.98,9.32 and 6.51 mg kg^{-1} respectively) while available Fe was in the deficient range (3.34 mg kg^{-1}) Table.1.

The details of the field experiments carried out and methods of analysis of soil and plant samples and the methodology followed in the refinement of prescription equations are presented below. The methodology adopted in this study is

Table 1. Initial soil characteristics of the experimental field

Soil pH	9.02
Soil EC (dS m^{-1})	1.15
Soil Texture	Sandy loam
Organic carbon (%)	0.41
Available –N (kg ha^{-1})	224
Available –P (kg ha^{-1})	22.7
Available –K (kg ha^{-1})	249.7
Cation Exchange Capacity (c. mol (p^+) kg^{-1}	27.4
Exchangable Sodium (m.eq/100 g of soil)	14.45
Exchangable Sodium Percentage (ESP)	52.73

the prescription procedure outlined by Truog (1960) and modified by Ramamoorthy *et al.* (1967) as "Inductive cum Targeted yield model" which provides a scientific basis for balanced fertilization and balance between applied nutrients and soil available nutrients forms. The field experiment with rice crop var. CO43Sub1 was conducted on sodic Vertisol in a randomized block design and was replicated thrice. Treatments were imposed based on initial soil test values (STVs) using the existing FPEs for Kalathur soil series. Based on the initial soil test values of available N, P and K and the quantities of N, P_2O_5 and K_2O supplied through FYM, fertilizer doses were calculated and applied for STCR treatments for various yield targets.

There were eight fertilizer treatments along with one control which were randomized in each strip in such a way that all the treatments occurred in both directions. Routine cultural operations were followed periodically. The sources of nutrients used in treatments were urea, single super phosphate and muriate of potash. The crop was grown to maturity, harvested and plot wise grain and straw yield was recorded. The grain and straw samples, post-harvest soil samples were collected from each plot. The soil and plant samples were processed and analyzed and NPK uptake by grain and straw samples were computed using the dry matter yield.

Basic parameters for fertilizer prescription equations

Making use of data on the yield of paddy, total uptake of N, P and K, initial soil test values for available N, P and K and doses of fertilizer N, P_2O_5 and K_2O applied, the basic parameters viz., nutrient requirement (NR), contribution of nutrients from soil (Cs), fertilizer (Cf) and farmyard manure (Cfym) were calculated as outlined by Ramamoorthy *et al.* (1967).

a. Nutrient Requirement (NR) is kg of N/ P_2O_5/ K_2O required per quintal (100 kg) of seed cotton production, expressed in (kg q^{-1}).

$$NR = \frac{\text{Total uptake of N or } P_2\ O_5 \text{or } K_2O \text{ (kg ha}^{-1}\text{)}}{\text{Seed cotton yield (q ha}^{-1}\text{)}} \quad (1)$$

b. Per cent contribution of nutrients from soil to total nutrient uptake (Cs):

$$Cs = \frac{\text{Total uptake of N or } P_2\ O_5 \text{or } K_2O \text{ in control plot (kg ha}^{-1}\text{)}}{\text{Soil test value for available N or } P_2\ O_5 \text{or } K_2O \text{ in control plot (kg ha}^{-1}\text{)}} \times 100 \quad (2)$$

c. Per cent contribution of nutrients from fertilizer to total uptake (Cf) (*see* eq. 3

d. Percent contribution of nutrients from organics to total uptake (Co)

Percent contribution from FYM (Cfym) (*see* eq. 4)

These parameters were used for developing fertilizer prescription equations for deriving fertilizers doses, and the soil test-based fertilizer recommendations were prescribed in the form of a ready table for desired yield target of cotton under NPK alone as well as under IPNS.

Fertilizer prescription equations

Making use of these parameters, the fertilizer prescription equations (FPEs) were refined for rice under sodic soil as furnished below.

$$Cf = \frac{\text{(Total uptake of N or } P_2\ O_5 \text{or } K_2O \text{ in treated plot (kg ha}^{-1}\text{)} - \text{soil test value for available N or } P_2\ O_5 \text{or } K_2O \text{ in control plot (kg ha}^{-1}\text{))} \times \text{AverageCs]}}{Fertilizer \text{ N or } P_2\ O_5 \text{or } K_2O \text{ applied}} \times 100 \quad (3)$$

$$Cfym = \frac{\textit{Total uptake of N or P or K in FYM treated plot}\text{(kg ha}^{-1}\text{)} - \text{soil test value for available N or P or K in FYM treated plot (kg ha}^{-1}\text{)} \times \text{Average Cs}}{Nutrient \text{ N/P/K added through FYM (kg ha}^{-1}\text{)}} \times 100 \quad (4)$$

Fertilizer nitrogen (FN)

$$FN = \left[\frac{NR}{(\frac{Cf}{100})} \times T\right] - \left[\frac{Cs}{Cf} \times SN\right] \quad (5)$$

$$FN = \left[\frac{NR}{(\frac{Cf}{100})} \times T\right] - \left[\frac{Cs}{Cf} \times SN\right] - \left[\frac{Cfym}{Cf} \times ON\right] \quad (6)$$

Fertilizer phosphorus (FP_2O_5)

$$FP_2\,O_5 = \left[\frac{NR}{(\frac{Cf}{100})} \times T\right] - \left[\frac{Cs}{Cf} \times 2.29SP\right] \quad (7)$$

$$FP_2\,O_5 = \left[\frac{NR}{(\frac{Cf}{100})} \times T\right] - \left[\frac{Cs}{Cf} \times 2.29SP\right] - \left[\frac{Cfym}{Cf} \times 2.29OP\right] \quad (8)$$

Fertilizer potassium (FK_2O)

$$FK_2\,O = \left[\frac{NR}{(\frac{Cf}{100})} \times T\right] - \left[\frac{Cs}{Cf} \times 1.21SK\right] \quad (9)$$

$$FP_2\,O_5 = \left[\frac{NR}{(\frac{Cf}{100})} \times T\right] - \left[\frac{Cs}{Cf} \times 2.29SK\right] - \left[\frac{Cfym}{Cf} \times 1.21OK\right] \quad (10)$$

where, FN, FP_2O_5 and FK_2O are fertilizer N, P_2O_5 and K_2O in kg ha^{-1}, respectively; NR is nutrient requirement (N or P_2O_5 or and K_2O) in kg q^{-1} , Cs is per cent contribution of nutrients from soil, Cf is per cent contribution of nutrients from fertilizer, Cfym is percent contribution of nutrients from FYM, T is the yield target in q ha^{-1}; SN,SP and SK respectively are alkaline $KMnO_4$-N, Olsen-P and NH_4OAc-K in kg ha^{-1} and ON, OP and OK are the quantities of N, P and K supplied through FYM in kg ha^{-1}. These equations serve as a basis for predicting fertilizer doses for specific yield targets (T) of rice under sodic condition for varied soil available nutrient levels.

Results

The basic parameters *viz.*, nutrient requirement (NR), contribution of nutrients from soil (Cs), fertilizer (Cf) and farmyard manure (Cfym) were computed and presented below.

Response of rice to fertilizer nutrients (Table 2)

Response of rice to different doses of fertilizer N, P_2O_5 and K_2O was assessed. The response varied from 843 kg ha^{-1} in FYM @ 6.25 Mg ha^{-1} to 2933 kg in STCR-IPNS-6.5 Mg ha^{-1}. The data showed that irrespective of STCR-NPK alone or STCR-IPNS, there was a progressive increase in response from lower target to higher targets and the magnitude of response was higher under STCR-IPNS than under STCR-NPK alone treatments. This formed the basis to compute the basic parameters and develop the fertiliser prescription equations under IPNS for rice under sodic soil.

Using the data on the grain yield, total uptake of N, P and K, initial soil test values for available N, P and K and doses of fertilizer N, P_2O_5, K_2O and FYM applied, the basic parameters *viz.*, nutrient requirement (NR), contribution of nutrients from soil (Cs), fertilizer (Cf) and farmyard manure (Cfym) were computed (Table 3).

Table 2. Response of rice (*var.*CO 43 Sub 1) as influenced by various treatments.

Treatments	Fertiliser doses (kg ha^{-1})			Mean grain yield (kg ha^{-1})	Response (kg ha^{-1})
	FN	FP_2O_5	FK_2O		
1 STCR- NPK alone – 5.5 Mg ha^{-1}	144	63	73	4878	2245
2 STCR- NPK alone – 6.0 Mg ha^{-1}	170	72	75**	5123	2490
3 STCR - NPK alone – 6.5 Mg ha^{-1}	197	75**	75**	5390	2757
4 STCR - IPNS – 5.5 Mg ha^{-1}	116	45	25*	5144	2511
5 STCR - IPNS – 6.0 Mg ha^{-1}	143	54	35	5371	2738
6 STCR – IPNS – 6.5 Mg ha^{-1}	170	64	49	5566	2933
7 FYM alone @ 6.25 Mg ha^{-1}	23	10	21	3476	843
8 FYM alone @ 12.5 Mg ha^{-1}	46	19	42	3987	1354
9 Absolute control	0	0	0	2633	0

Where, *maintenance dose; ** maximum dose

Table 3. Experimental data for refinement of fertilizer prescription equations for rice (*var. CO 43 Sub1*) under Sodic soil (Mean of three replications)

Treatment	Grain yield	Straw yield	UN	UP	UK	SN	SP	SK	FN	FP_2O_5	FK_2O	FYM ($Mg\ ha^{-1}$)
	($kg\ ha^{-1}$)											
STCR- NPK alone – 5.5 $Mg\ ha^{-1}$	4878	6342	71.53	15.24	82.85	229	16.9	250	144	63	73	0
STCR- NPK alone – 6.0 $Mg\ ha^{-1}$	5123	7255	73.27	16.47	84.51	231	19.0	248	170	72	75**	0
STCR - NPK alone – 6.5 $Mg\ ha^{-1}$	5390	7135	75.84	15.14	84.90	229	18.4	251	197	75**	75**	0
STCR - IPNS – 5.5 $Mg\ ha^{-1}$	5144	7133	74.24	14.05	84.24	230	17.3	251	116	45	25*	12.5
STCR - IPNS – 6.0 $Mg\ ha^{-1}$	5371	7163	80.74	18.38	87.27	231	18.2	250	143	54	35	12.5
STCR – IPNS – 6.5 $Mg\ ha^{-1}$	5566	7285	82.31	19.43	92.59	231	17.3	251	170	64	49	12.5
FYM alone @ 6.25 $Mg\ ha^{-1}$	3476	5284	44.5	7.47	68.65	231	21.9	246	23	10	21	6.25
FYM alone @ 12.5 $Mg\ ha^{-1}$	3987	5672	45.83	8.72	69.67	233	22.5	248	46	19	42	12.5
Absolute control	2633	4894	48.51	9.92	60.65	225	24.5	226	0	0	0	0
Range	2633-5566	4894-285	44.50-82.31	7.47-19.43	60.65-92.59	225-233	16.9-24.5	226-251				
Mean	4619	6463	66.31	13.87	79.48	230	19.56	247				

Where, *maintenance dose; ** maximum dose

Basic parameters (Table 4)

Using the pre-sowing soil available N, P and K, fertiliser doses applied, grain yield and total N, P and K uptake, the basic parameters *viz .,* nutrient requirement(NR) contribution of nutrients from soil (Cs), fertilisers (Cf) and FYM (Cfym) were computed. The results emanated from the present investigation revealed that Rice *var.* CO 43 Sub1 requires 1.44 kg N, 0.29 kg P_2O_5 and 1.76 kg K_2O for producing one quintal of grain yield. The percent contribution of N from soil was 17.18 and fertiliser was 23.71. With regard to P_2O_5, the percent contribution from soil was 28.14 and fertiliser was 18.2 while for K_2O, the percent contribution from soil was 23.42 and from fertiliser was 59.4 percent. The percent contribution of nutrients from FYM was 18.89, 12.56 and 43.59 per cent N, $P_2O_5$and K_2O, respectively.

Table 4. Basic parameters for rice *(var.CO 43 Sub1)*

Parameters	Basic data		
	N	P_2O_5	K_2O
Nutrient requirement (kg q^{-1})	1.44	0.29	1.76
Per cent contribution from soil (Cs)	17.18	28.14	23.42
Per cent contribution from fertilizers (Cf)	23.71	18.2	59.4
Per cent contribution from FYM (Cfym)	18.89	12.56	43.59

Fertiliser prescription equations for rice under sodic soil

Making use of the basic parameters *viz*., NR, Cs, Cf and Cfym, the Fertiliser Prescription Equations were developed under NPK alone and IPNS.

STCR-NPK alone	STCR-IPNS (NPK+FYM)
FN = 6.08 T- 0.72 SN	FN = 6.08 T - 0.72 SN- 0.80 ON
FP_2O_5 = 1.64 T- 1.55 SP	FP_2O_5 = 1.64 T -1.55 SP- 0.69OP
FK_2O = 2.96 T- 0.39 SK	FK_20= 2.96 T - 0.39 SK- 0.73OK

where, FN, FP_2O_5 and FK_2O are fertiliser N, P_2O_5 and K_2O in kg ha^{-1}, respectively; T is the grain yield target in q ha^{-1} and SN, SP and SK respectively are alkaline $KMnO_4$–N,Olsen–P and NH_4OAc –K in kg ha^{-1}; ON, OP and OK are quantities of N, P and K in kg ha^{-1}supplied through FYM.

Discussion

There was an increase response of N, P_2O_5 and K_2O observed with increase in fertilizer levels. The highest response ratio (RR) of N, P_2O_5 and K_2O observed in STCR -IPNS treatments. This was in line with the findings of (Veeranna and Srijaya, 2017) who reported that increased level of nitrogen and phosphorous had a significant effect on growth and yield in maize. This was observed for rice also.

Ready reckoner of fertilizer doses (nomograms) was formulated for desired yield targets of rice *var*. CO 43 Sub1 for a range of soil test values under IPNS (NPK plus FYM @ 12.5 t ha^{-1}). Using the fertiliser prescription equations under IPNS, the extent of saving of inorganic fertilizers was computed. Therefore, under IPNS (NPK+FYM@12.5 t ha^{-1}), 42, 18 and 32 kg of fertilizer N, P_2O_5 and K_2O respectively could be reduced from the recommended dose of fertilizers for a specific soil test value and yield target resulting in economy of fertilizer use. Therefore, the integrated use of FYM along with NPK fertilizer not only maximized of yield but also accelerated the profitability. It also improved the soil fertility by the way of increased microbial population and also favorable soil physical properties, thereby conserving the soil fertility for long time. In the present study also, these factors contributed the improvement in yield of rice under sodic soil by the integrated use of NPK along with FYM. In recent times, the fertilizer recommendations derived from STCR approaches may be more appropriate than other approaches Santhi et al., (2017).

A perusal of the estimate showed that when NPK alone were applied, for a soil test value of 180 kg $KMnO_4$-N ha^{-1}, the doses of fertilizer N required for desired yield targets of 5.5, 6.0 and 6.5 Mg ha^{-1}were 204, 235 and 265 kg ha^{-1}, respectively. When FYM @ 12.5 Mg ha^{-1} was applied along with NPK, the required fertilizer N doses were 162, 193 and 223 kg ha^{-1} with 20.58, 17.87 and 15.84 per cent reduction in fertilizer doses over NPK alone respectively for the same yield target and soil test values. In case of P fertilization, when NPK alone were applied, for a soil test value of 18 kg ha^{-1} of Olsen-P, the doses of fertilizer P_2O_5 required for desired yield targets of 5.5, 6.0 and 6.5 Mg ha^{-1} were 62, 70 and 78 kg ha^{-1} respectively. When NPK were applied along with FYM @ 12.5 Mg ha^{-1}, the doses were 44, 52

Table 5. Estimates of soil test based fertilizer doses (kg ha^{-1}) for desired yield targets of Paddy under sodic soil

Soil test values (kg ha^{-1})			NPK alone									NPK +FYM @ 12.5 Mg ha^{-1}								
			5.5 (Mg ha^{-1})			6.0 (Mg ha^{-1})			6.5 (Mg ha^{-1})			5.5 (Mg ha^{-1})			6.0 (Mg ha^{-1})			6.5 (Mg ha^{-1})		
SN	SP	SK	FN	FP_2O_5	FK_2O	FN	FP_2O_5	FK_2O	FN	FP_2O_5	FK_2O	FN	FP_2O_5	FK_2O	FN	FP_2O_5	FK_2O	FN	FP_2O_5	FK_2O
180	18	200	204	62	84	235	70	99	265	78	114	162	44	52	193	52	67	223	60	75**
220	22	240	176	56	69	206	64	84	236	72	98	134	38	37	164	46	52	194	54	66
240	24	260	161	53	61	192	61	76	222	69	91	119	35	29	150	43	44	180	51	59
260	26	280	147	49	53	177	58	68	208	66	83	105	31	25*	135	40	36	166	48	51

NB: * maintenance dose. **maximum dose

Blanket dose: 150:50:50 kg ha^{-1} of fertiliser N, P_2O_5 & K_2O respectively for rice (*var.CO 43 Sub1*).

and 60 kg ha^{-1} with a per cent reduction of 29.03, 25.71 and 23.07 respectively over NPK alone. With regard to fertilizer K_2O doses, it was found that to achieve the desired yield targets of 5.5, 6.0 and 6.5 Mg ha^{-1}, for a soil test value of 200 kg NH_4OAc-K, the fertiliser K_2O requirements were 84, 99 and 114 kg ha^{-1}, respectively. When NPK were applied with FYM @ 12.5 Mg ha^{-1}, the K_2O doses were 52, 67 and 82 kg ha^{-1}with a percent reduction of 38.09, 32.32 and 28.07 over NPK alone, respectively. The targeted yield equations based on soil tests will not only ensure sustainable crop production but also steer the farmers towards economic use of costly fertilizer inputs (Tegegnework *et al.,* 2015). These findings are in conformity with those reported by (Suresh and Santhi 2017) on Alfisol and by Udayakumar and Santhi (2017) on Inceptisol in pearl millet. Srivastava et al., (2017) and Vedhika Sahu *et al.,* (2017) reported the enhanced yield of rice on Vertisol of Chhattisgarh.

Conclusions

In the present investigation, Soil Test Crop Response (STCR) based Integrated Plant Nutrition System (IPNS) for desired yield targets of rice e has been developed for deep black calcareous, Allathur soil series Vertic Ustropept, hyper thermic family of sodic Natustalf exhibiting sandy loam texture soils of Tamil Nadu. The targeted yield approach of fertilizer prescription ensures nutrient balancing to suit desired yield targets based on resource availability of the farmer with sustained soil fertility. It was also found that the application of STCR-IPNS technology along with FYM @ 12.5 Mg ha-1 (with 30% moisture, 0.60%, 0.30% and 0.5% NPK, respectively), there was a saving of 42, 18 and 32 kg of fertiliser N, P_2O_5 and K_2O, respectively.

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Transforming Transient Drain Spacing Formula to Predict Water Table Fluctuation in Response to Constant Recharge

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Abstract

A method was developed for transforming transient drain spacing equations for predicting water table heights in response to constant rainfall without ignoring flow above drain level between parallel subsurface drains. The Integrated Hooghoudt drain spacing equation was transformed for predicting rise and fall of water table heights in response to constant recharge. The equation was tested in subsurface drained fields with drain spacing of 50 m and 75 m and compared with existing drain equations. Average per cent deviations of predicted water table heights by Integrated Hooghoudt equation with instantaneous rise, Integrated Hooghoudt with rise factor, van de Leur Maasland, van Schilfgaarde and de Zeeuw Hellinga equations were 25.72, 16.71, 27.38, 27.20 and 24.50% with corresponding RMSD of 0.2083, 0.2110, 0.1663, 0.2020 and 0.1769, respectively for 50 m drain spacing plot and 15.90, 22.56, -28.54, -14.57 and 14.39% with corresponding RMSD of 0.1779, 0.2070, 0.2187, 0.1226 and 0.0911, respectively for 75 m drain spacing plot. The approach for transforming a transient drain spacing equation to water table predicting equation in response to constant recharge is validated.

Key words: Drain spacing equations, Subsurface drainage, Salinity, Waterlogging

Introduction

Extensive waterlogging and salinization occurred in almost every largescale irrigation project, where due importance to drainage was not given. Waterlogging may be defined as stagnation of water on the land surface or where the water table rises to an extent that soil pores in the crop root zone become saturated, resulting restriction in normal circulation of air leading to decline in the level of oxygen and increase in the level of carbon dioxide (Settler *et al.*, 2009). The major reasons of waterlogging and salinization in irrigated agriculture are excessive seepage from canal, over irrigation, introduction of high-water demanding crops, obstruction of natural drainage, entry of runoff water from adjoining area, high rainfall and inadequate drainage provisions. Secondary salinization is associated with waterlogging in arid and semi-arid regions where annual precipitation is much less than the annual rainfall. Much of the world's saline land is subjected to the waterlogging (Qureshi and Barrett- Lennard, 1998). Waterlogging results in poor aeration of root zone causing a condition of hypoxia (low oxygen concentrations) as most of the pores are filled with water and no space left for air. In addition, waterlogging can cause the accumulation of ethylene and products of root and bacterial anaerobic metabolites (carbon dioxide, ethanol, lactate, etc.). Nutrients such as iron changes to its reduced conditions and become toxic to the crops. Microbial activity gets reduced due to low temperature hence mineralization rate is also reduced making slow release of nutrients from FYM and compost. Low soil temperature causes slow germination and there exists high risks of disease. Use of tillage machineries becomes difficult under high moisture conditions in the field.

Subsurface flow of any region increases putting additional loads on natural subsurface drainage system of the area when irrigation is introduced without due consideration to drainage. In case load due to subsurface flow is much higher than the natural subsurface flow capacity of the region water table starts rising and waterlogging occurs. Hydrology of the region gets upset. Generally, in dry regions (arid and semiarid) subsurface drainage capacity of the subsoil is not high as nature has not evolved itself to cope up with high rainfall or inflow of water waterlogging is more prevalent. Changes in land use and irrigation development always upset the natural hydrological balance of the region (Heuperman *et al.,* 2002). The threat of soil and groundwater salinization induced by irrigation has become a major issue for agriculturists, researchers, hydrologists and agricultural, irrigation and drainage scientists. Waterlogging adversely affects crop productivity in about 4.7 M ha irrigated soils of the Indo-Gangetic Plains of North India alone. Nearly 2.5 M ha of area is affected with sodicity and 2.2 M ha with excessive seepage from canals (Minhas and Dagar, 2007; Singh, 2009).

A great effort had been made worldwide during the last 50 years for increasing the availability of irrigation water to sustain the agricultural productivity and eliminate hunger. In this period, the net canal irrigation potential has increased from 95 M ha to 260 M ha in the world and from 22.5 M ha to 57 M ha in India. About 4.7 M ha of irrigated soils of Indo-Gangetic Plains has 2.2 M ha seepage affected from unlined canals and 2.5 M ha due to sodicity (Minhas and Dagar, 2007; Singh, 2009). Salinization is a global problem and it affects about 20-30 M ha of the world's 260 M ha of irrigated land (FAO, 2000). Irrigated agriculture is developing over the globe with continuous increase in waterlogging and salinization of agricultural field (Valipour, 2014a). In India alone, nearly 1.79 M ha of productive agricultural is suffering due to severe seasonal and perennial waterlogging (Sharma *et al*., 2009) and 6.75 M ha due to salt build up (Sharma and Gupta, 2010). Current drainage improvement rate is less than 0.5 million ha per year which is insufficient to balance the current growth of affected drainage areas. Less than 20% of the global area is drained, emphasizing the need of drainage for controlling waterlogging and salinity (FAO, 2013; IDD, 2013). Only 10%-20% of the irrigated land is equipped with drainage while 40%-60% is in need of drainage (Smedema *et al*., 2000). Agenda 21 of Earth Summit stresses the need for drainage as a necessary complement to irrigation development in arid and semi-arid areas (UNCED, 1992). Pressure on irrigated agriculture is mounting because of reduction in net sown area due to excessive waterlogging and salinization, urbanization, industrializations, decreasing fertility status of the fields and various developmental activities. Ultimately more pressure on irrigated agriculture is being experienced for increasing food grain production which can be met out by reclaiming waterlogged, waterlogged sodic and waterlogged saline soil for crop production.

Lowering of water table is one of the important basic necessities for the reclamation of saline and alkaline soils in arid and semi-arid regions. Subsurface drainage is a well-recognized engineering measure for lowering water table of waterlogged salt affected soils for increasing agricultural productivity. Research on subsurface drainage is still continued to tackle various associated problems (Guedessou *et al*., 2021). The design of drainage system for controlling salinity and excess moisture is based on the control of water table position for a given hydraulic and hydrologic situation. Information on water table fluctuation is the most important for correlating crop yield and drainage performance and also optimizing the drain spacing. Large numbers of steady state drain spacing formulas (Donan, (1946); Ernst (1956 and 1962); Hooghoudt, (1940), Kirkham (1958), Dagan (1964), van Beers (1979) and transient formulas (Glover (1954), Dumm (1960), Inegrated Hooghoudt and Integrated Toksoz-Kirkham (1961), van Schilfgaarde (1963), Modified Glover by van Schilfgaarde (1964), Integrated Dagan by Chhedi Lal & Singh (2002) are available for drain spacing calculation. A large number of mathematical models are also available for predicting water table fluctuation in response to recharge (Wiser *et al*. (1974), Kraijenhoff van de Leur (1958), Maasland (1959), de Zeeuw and Hellinga (1958), Dumm (1960), van Schilfgaarde (1965) and Skaggs (1982)). Kraijenhoff van de Leur (1958) and Maasland (1959) derived identical theoretical equations independently for simulation of water table fluctuation between two subsurface drains in response to various recharge patterns. Uzaic and Chieng (1988) derived drain spacing formula having elliptical shape of water table. Dumm (1960) drain spacing formula had been also used for predicting water table fluctuations assuming instantaneous rise in water table due to constant recharge. These models can be used to predict water table heights for any length of time. de Zeeuw and Hellinga (1958) and van Schilfgaarde (1965) transformed the Hooghoudt's drain spacing formula and Kirkham's drain spacing formula using an empirical relationship to predict transient water table behavior in response to constant recharge on daily basis, respectively. Efforts are continuously being made to develop model for predicting water table fluctuations in response to recharge from time to time for their extended applications. Dang *et al*. (2010) studied modeling of drainage and ground water table above the collecting pipe through 2-D ground water model for leachate analysis. Valipour (2012) made a comparison between horizontal and vertical drainage in anisotropic soil considering water table fluctuations between drains and wells using EnDrainWin and WellDrain models. Singh (2010) gave generalized analytical solutions for water table in inclined aquifers in the presence of subsurface drains. Hornbuckle *et al*. (2005) managed controlled water table as a strategy for reducing salt loads from subsurface drainage under perennial agriculture and showed that controlled drainage significantly reduced salt loads and drainage volumes compared to unmanaged systems. Marked increase in soil salinity needed to be carefully managed. Castanheira and Santos (2009) presented a numerical model for predicting water table height in subsurface drainage. The results obtained compared well with total head calculated by classical Khirkam's and Hooghoudt analytical solutions. Rosa *et al*. (2002) evaluated WATABLE model for field applications of sub-irrigation of potato crop. They optimized the water table depth for growth stage, and duration, frequency, and rate of irrigation. Singh and Jaiswal (2010) developed a model for water table fluctuation in the response to a time-varying exponential recharge and depth-dependent ET in a two-dimensional aquifer system with an inclined base. Singh *et al*. (2007) solved linearized Boussinesq equation and derived an analytical solution for predicting the water table fluctuation in subsurface drained field in presence of recharge and evapotranspiration (ET). Kumar *et al*. (2012) tested transient drain spacing formula in the field and found modified Glover as best performing. Sanjayan and Rajan (2015) found good agreement between subsurface drain flows and water table height predicted by DRAINMOD and observed in fields of cold season potato field. DRAINMOD model despite successful calibration and validation results in the sugarcane fields faces adoption challenges (Malota and Senzanje 2015). Sontakke1 and Rokade (2014) gave a numerical solution of a 1-D linearized Boussinesq's equation to predict the water table fluctuations in an unconfined aquifer due to time varying recharge from recharge (rectangular) basin. Water table fluctuation is an essential parameter for designing drain spacing and testing the efficacy and performance of subsurface drainage system. For optimization of subsurface drain spacing long term crop based water table fluctuation data are required. Available numerical and analytical models have their own limitations. Most of the analytical solutions for predicting water table fluctuations do not consider flow above drain level and may predict higher value of water table height if flow above drain level is significant. van Schilfgaarde's model (1965) requires no correction for radial flow but ignores flow above drain axis. Similarly, de Zeeuw- Hellinga model requires radial flow correction and also ignores flow above drain level. In the present study a new methodology is proposed to transform any transient drain spacing formula for predicting water table fluctuations in response to rain or irrigations. Integrated Hooghoudt (van Schilfgaarde, 1963) transient drain spacing formula which takes flow above drain level also into account has been transformed to predict water table fluctuation in response to constant intermittent recharge by proposed method and tested with field observations of 50 m and 75 m drain spacing water table fluctuation data.

Material and Methods

Classical models

Different theoretical approaches for prediction of water table height above drain level have been used by various investigators under certain assumptions and approximations. The solution of Kraijenhoff van de Leur-Massland and van Schilfgaarde have been compared in the present study. de Zeeuw and Hellinga (1958) stated that the change in drain discharge is proportional to the excess recharge (R-q) with proportionality constant as reaction factor (α) for constant recharge (R) in a given time interval.

$$\frac{dq}{dt} = \alpha \ (R - q) \tag{1}$$

Upon integration between time limits t=t, $q=q_t$ and t=t-1, $q = q_{t-1}$ results.

$$q_t = q_{t-1} e^{-\alpha \Delta t} + R\left(1 - e^{-\alpha \Delta t}\right) \tag{2}$$

Where,

q_t = drain discharge rate at time t=t,

q_{t-1} =drain discharge rate at time t=t-1,

Δt = t- (t-1), the time interval

R = constant recharge or rainfall

Water table height was simulated by steady state drain spacing formula of Hooghoudt (1940) $(q = \frac{8Kd}{L^2} h)$ ignoring flow above drain level as below.

$$h_t = h_{t-1} e^{-\alpha \Delta t} + \frac{R}{0.8 f \alpha}\left(1 - e^{-\alpha \Delta t}\right) \tag{3}$$

Where,

h_t = water table height at time t=t,

h_{t-1} = water table height at time t=t-1,

Δt = t- (t-1), the time interval

f = drainable porosity

$\alpha = \frac{\pi^2 Kd}{fS^2}$, reaction factor

Equation (3) accounts for radial flow but does not take the flow above drain level into account.

Kraijenhoff van de Leur (1958) and Massland (1959) derived independently a solution for predicting water table height which is based on steady state recharge over any period, t instead of an instantaneous rise in water table height due to any recharge as assumed by Glover (Dumm, 1954). The following differential equation for non-steady flow has been utilized by Dumm (1960).

$$Kd \frac{\partial^2 h}{\partial x^2} = f \frac{\partial h}{\partial t} - R \tag{4}$$

under the following boundary conditions

h = 0, for t = 0 and 0 < x < S (initially horizontal water table at drain level)

h = 0, for t > 0 and at x = 0, x = S (water table at drains at zero level)

R = constant recharge rate for t > 0

The height of water table midway between the parallel drains (x = ½ S) at any time t, was obtained as,

$$h_t = \frac{4}{\pi} \frac{R}{f} j \sum_{n=1,-3,5,\ldots}^{\infty} \frac{1}{n^3}\left(1 - e^{-n^2 t/j}\right) \tag{5}$$

Where,

d = depth of impermeable layer below drain level, m

D = (d+1/2 h_0), average depth of aquifer, m

F = drainable porosity expressed as a fraction of the soil volume and assumed constant for a given soil, dimensionless

h_0 = initial water table height midway between the drains above drain level, m

h_t = water table height midway between the drains above drain level at any time t, m

j = fS^2/π^2 KD, termed as reservoir coefficient, d

K = saturated hydraulic conductivity, m/d

R = constant recharge rate, m/d

S = spacing between the drains, m

The use of average depth of aquifer D in the formula would yield quite erroneous results. Further this assumption does not take radial flow into account. Depth to impermeable layer below drain axis can be replaced by equivalent depth of impermeable layer of Hooghoudt (1940), which accounts for radial flow near drains. Flow above drain level can be accounted in average thickness of aquifer D, but the result may not be realistic. The equation can be used in a more appropriate way only after neglecting flow above drain level and accounting for radial flow near the drains.

van Schilfgaarde (1965) proposed a model similar to Zeeuw and Hellinga model for prediction of water table behavior, from long term rainfall or irrigation records, for developing optimized design of subsurface drainage system using Kirkham (1958) steady state drainage solution. The water table height at the end of any time "t" is given by,

$$h_t = \left[h_{t-1} + \frac{A}{f}\left(e^{-\frac{1}{A}} - 1 \right)(EXS_t) \right] e^{-\frac{1}{A}} \quad (6)$$

Where,

A = f F C S/K, a parameter with dimensions of time which describes both the geometry of the system as well as soil properties, d

C = correction or shape factor introduced by Bouwer and van Schilfgaarde (1963)

F = an infinite series defined by Toksoz and Kirkham (1961) which is a function of drain diameter, spacing and depth of impervious layer below drains axis given as below,

$$F = \frac{1}{\pi}\left[\ln\frac{1}{\pi r} + \sum_{n=1,\ldots}^{\infty} \frac{1}{n}\left(\cos\frac{2n\pi r}{S} - \cos n\pi \right)\left(\coth\frac{2n\pi d}{S} - 1 \right) \right] \quad (7)$$

h_t = water table height at any time t, m

h_{t-1} = water table height at time t-1, m

r = radius of the drain, m

EXS_t = (SMC_t – MSC), input to drainage system during time t, m

SMC_t = soil moisture content at the end of time t, m

MSC = maximum soil moisture storage capacity, m

SMC_t may be calculated from the following relationship,

$$SMC_t = SMC_{t-1} + P_t + I_t - ET_t \quad (8)$$

$SMC_t \leq$ MSC, for all t

SMC_{t-1} = soil moisture content at the end of time t-1, m

P_t = (rainfall – surface runoff), net precipitation during time t, m

I_t = irrigation amount during time t, m

ET_t = evapotranspiration amount during time t, m

Equation (4) was introduced by Wiser and van Schilfgaarde (1964). If the sum on right hand side in equation (4) is greater than MSC, the amount of excess EXS_t , is taken as percolation downward to water table and SMC_t is set equal to MSC.

Theoretical development

de Zeeuw and Hellinga (1958) and Bouwer and van Schilfgaarde (1963) assumed that the instantaneous drainage rate midway between the drains could be taken equal to the steady state drainage rate corresponding to the same water table height between the drains above the drain axis. Thus they used the steady state drainage relationship to describe the rate of fall of water table midway between the drains by introducing a correction factor C in the equation given below,

$$q = -fC\frac{dh}{dt} \quad (9)$$

Where,

q = steady state drain discharge rate per unit area, m/d

-ve sign here, denotes h decreasing with increasing t. Correction factor C was assigned following values,

$0.02 < h/s < 0.08$, C = 0.08

$h/s > 0.15$, C = 1.00

replacing q from Hooghoudt (1940) steady state drainage relationship and integrating for t = 0, $h_t = h_0$ and t = t, $h_t = h_t$, they obtained following relationship for falling water table condition.

$$S = \left[\frac{8Kd_e t}{Cf \ln\frac{h_0(2d_e + h_t)}{h_t(2d_e + h_0)}} \right]^{\frac{1}{2}} \quad (10)$$

Where,

d_e = equivalent depth of impervious layer below drain level after Hooghoudt (1940), m

Above equation may also be rearranged as,

$$h_t = \frac{2d_e}{\left(\frac{2d_e + h_0}{h_0}\right)\exp\left(\frac{8Kd_e t}{CfS^2}\right) - 1} \quad (11)$$

Originally Equation (10) did not take the radial flow into account. The same was accounted by replacing depth of impervious layer with equivalent depth of impervious layer. The equation considers both flow above and below drain level.

From field experience and various drainage theories following points were observed.

1. Rate of water table rise is directly proportional to effective recharge, R and inversely proportional to drainable porosity, f and correction factor, C (from Equation 9).
2. Rate of water table rise is directly proportional to water table build up constant, WB_c and inversely proportional to actual reaction factor, a.

Equation can be modified taking above points into account.

$$\frac{dh}{dt} = \frac{RWB_c}{C.f.s} \quad (12)$$

Falling rate constant and water table build up constant

For understanding falling rate constant, one may consider the basic drainage equation of Dumm (1960).

$$h_t = 1.16\, h_0 e^{\alpha.t} \quad (13)$$

Where,

$$\alpha = reaction\ factor = \frac{\pi^2 K d_e}{fS^2}, d^{-1}$$

Water table height at time t_1 will be,

$$h_1 = 1.16\, h_0 e^{\alpha t_1} \quad (14)$$

and at time t_2 will be,

$$h_1 = 1.16\, h_0 e^{\alpha t_2} \quad (15)$$

Taking ratio of Equation (11) and (10),

$$\frac{h_2}{h_1} = e^{-\alpha(t_2 - t_1)}$$

$$\frac{h_2}{h_1} = e^{-\alpha\Delta t} \quad (16)$$

α for a given set of drains spacing is fixed and if Δt is taken as one day, Equation (16) reduces to,

$$\frac{h_2}{h_1} = \gamma_c \quad (17)$$

$$h_2 = h_1\gamma_c$$

The value of h_2/h_1 will be constant, which is termed as falling rate constant. If this rate is known, the water table height on next day may be calculated by multiplying water table height on previous day with falling rate constant. Above equation also indicates that the higher is the falling rate constant the faster would be the drop.

If the rate of drop is higher, the buildup of water table will be lower. This implies higher is the water table build up constant, the higher will be the rate of rise. Water table build up constant may be estimated as,

$$WB_c = 1 - \gamma_c \quad (18)$$

Actual reaction factor (a)

Taking the log of equation (16)

$$\log h_1 - \log h_2 = \alpha\Delta t \quad (19)$$

$$\alpha = \frac{\log h_1 - \log h_2}{\Delta t} \quad (20)$$

If Δt = 1 day, the equation reduces to,

$$\alpha = \log h_1 - \log h_2 \quad (21)$$

This is the slope of the curve obtained by plotting water table height on log scale and time on linear scale and is known as reaction factor.

This equation does not consider flow above drain level hence it does not reflect the correct conditions. If the water table heights of drained field for two consecutive or more days are known, the slope of the line may be termed as actual reaction factor (a) estimated from field observations. Equation (12) may also be written as,

$$\frac{\Delta h}{\Delta t} = \frac{R_{\Delta t}(1 - e^{-\alpha\Delta t})}{CfS} \quad (22)$$

where,

Δh = rise in water table height due to recharge in time Δt, m

$R_{\Delta t}$ = recharge during time interval, Δt,m

Δt = time interval, d

Equation (11) may be also written as,

$$h_t = \frac{2d_e}{\frac{2d_e + h_{t-1}}{h_{t-1}} \exp\left(\frac{8Kd_e}{CfS^2}\right)\Delta t - 1} \qquad (23)$$

A general solution for water table fluctuation can be written as,

$$h_t = (h_{t-1} - \Delta h_d) + \Delta h_R \qquad (24)$$

Where,

Δh_d = drop of water table in time t, due to drainage, m

Δh_R = rise in water table height due to recharge, m

Following the above logic and taking time interval Δt = 1 day and combining the equation (23) and (24), one gets,

$$h_t = \frac{2d_e}{\frac{2d_e + h_{t-1}}{h_{t-1}} \exp\left(\frac{8Kd_e}{CfS^2}\right) - 1} + \frac{R_{\Delta t}}{Cfa}\left(1 - e^{-a}\right) \qquad (25)$$

The above equation may be used for predicting water table height for any period of time but only through a successive procedure, provided the soil properties and effective recharges are known.

The above approach may be utilized for transforming any transient drainage solution for falling water table condition to predict water table behavior in response to constant recharge rate. The transformed model using the above approach will have the same limitations as that of the original Solutions.

Experimental Verification and Comparison with Other Models

Experimental area

The experimental area is located at Sampla at a longitude 76°46′ E and 18°45′ N latitude about 220 m above mean sea level in Rohtak district of Haryana State (India). The area receives an annual rainfall in the range of 500 to 600 mm and lies in semi arid region. The area is bowl shaped and due to flat terrain and unavailability of proper outlet, drainage congestion becomes quite acute in monsoon season. Soils are sandy loam in texture in upper 1.8 m layer and loamy sand below this layer. Analysis is based on the observations of drainage plot with 50 m and 75 m drain spacing. The soil hydrological parameters used in the different water table prediction models for the case are given in Table 1.

Experimental procedure

The rainfall data of Sampla for the year 1985 were analyzed using water balance model (Eqn. 8) for the month of July. The observations of rainfall and water table heights from 17 July to 31 July were considered for the purpose of comparison. First fifteen days were left for stabilizing of the drainage system and collecting appropriate data for the study purposes. The root zone was considered up to a depth of one meter with bulk density in this zone as 1.5 g/cm^3. For practical purpose the moisture at field capacity was taken equal to moisture holding capacity (SMC) i.e 20 percent (volume basis). The total moisture in terms of depth was calculated as a product of SMC and bulk density which was equal to 330 mm.

Table 1. Average values of soil hydrological parameters

Parameters	50 m spacing	75 m spacing	Unit
Saturated hydraulic conductivity, K	2.65	4.1	m/d
Depth to impervious layer below drain axis, d	1.2	1.2	m
Equvalent depth of impervious layer below drain axis, d_e	1.2	1.1976	m
Transmissivity			
Kd	3.18	4.91	m^2/d
Kd_e	3.18	4.92	m^2/d
Drainable porosity, f	0.14	0.14	dimensionless
Reaction factor, α	0.092546	0.063508	d^{-1}

Experimental area was bunded and any runoff crossing the boundary was ignored. Since the area was bunded surface runoff was neglected. Effective rainfall or drainage input was calculated making use of water balance model, which has been defined as the difference of SMC_t and MSC from daily water balance for the whole month. Water table height was recorded for the month of July, 1985 (Chhedi Lal, 1986). The measured water table height above drain axis on 16 July 1985 was 1.20 m for 50 m drain spacing and 0.95 m for 75 m drain spacing plots. A small computer programme in Fortran 77 was developed to solve the van de Leur–Massland Equation (5) whereas Transformed Integrated Hooghoudt Equation (25), van Schilfgaarde Equation (6) and de Zeeuw and Hellinga Equation (3) were solved with scientific calculator making use of the soil hydrological parameters and effective rainfall. Glover (1954), Dumm (1960) and Integrated Hooghoudt transient drain spacing equations were also solved for predicting water table heights assuming instantaneous rise of water table height due to recharge (R/f). Percent deviations of predicted water table heights with observed values were calculated for making comparison.

Results and Discussion

The observed values of water table height for continuous period of 15 days from July 17 to July 31, 1985 receiving 5 spells of effective rainfall from a subsurface drained plot of spacing 50 m and 75 m laid at 1.75 m depth were compared with the predicted values of water table heights by de Zeeuw and Hellinga Equation (DZHE), Kraijenhoff van de Leur- Massland Equation (KVDLME), van Schilfgaarde Equation (VSE) and Integrated Hooghoudt Equation With Rise Factor (IHEWRF), Integrated Hooghoudt Equation With Instantaneous Rise (IHEWIR) in order to study their field applicability. Glover Equation with Instantaneous Rise and with shape factor 1.27 (GEWIR), Glover Equation With Rise Factor and with shape factor 1.27 (GEWRF), Dumm Equation With Instantaneous Rise and with shape factor 1.16 (DEWIR), Dumm Equation With Rise Factor and with shape factor 1.16 (DEWRF), Glover-Dumm Equation With Instantaneous Rise and with shape factor one (GDEWIR) and Glover-Dumm With Rise Factor and with shape factor one (GDEWRF) were also tested for their relative superiority for field applications. Observed and predicted water table behaviors with time are shown in Fig. 1 & 2. Percentage deviations of predicted water table height by various equations are presented in Table 2.

50 m Drain spacing plot

It may be seen from Fig. 1 that GEWIR, GEWRF, DEWIR and DEWRF could not predict water table trend and indicated only rising trend. Their respective percent deviations ranged from 3.29 to -3188.99%, 3.03 to -13580.58%, 11.69 to -812.11% and 11.18 to -840.26% and corresponding mean per cent deviations of -890.75%, -905.37%,-277.16% and -285.58%, respectively. Root Mean Square Deviation (RMSD) of GEWIR, GEWRF, DEWIR and DEWRF were calculated as 5.6472, 5.7447, 1.7112 and 1.7644, respectively. GDEWIR and GDEWRF predicted water table heights which matched well with the values observed in the fields. The per cent deviations of predicted water table heights by GDEWIR and GDEWRF over observed values ranged from 3.34 to -40.87% and 1.72 to -53.15% with corresponding mean percent deviations of 20.11% and 24.31% and RMSD of 0.1729 and 0.1879, respectively.

It may be further seen from Fig. 1 that IHEWRF, IHEWIR, KVDLM, VSE and DZHE predicted water tables pretty well in 50 drain spacing plot. The percent deviations of predicted water table heights by IHEWRF, IHEWIR, KVDLM, VSE and DZHE over observed values ranged from 0.01 to 40.66%,-1.30 to 30.29%, 5.32 to -67.57%, -5.00 to -62.16% and 1.67 to -54.07 with correspond mean per cent deviations of 25.72%, 16.71%, 27.38%, 27.20% and 24.50% and RMSD of 0.2083, 0.2110, 0.1663, 0.2020 and 0.1769, respectively. The IHEWIR gave the lowest mean per cent deviations and RMSD values. All other equations with rise factor performed equally well with their order of performance as KVDLM, DZHE, VSE, IHEWRF and IHEWIR. GDEWIR and GDEWRF performed better than DZHE, IHEWRF, IHEWIR and VSE. Thus the overall

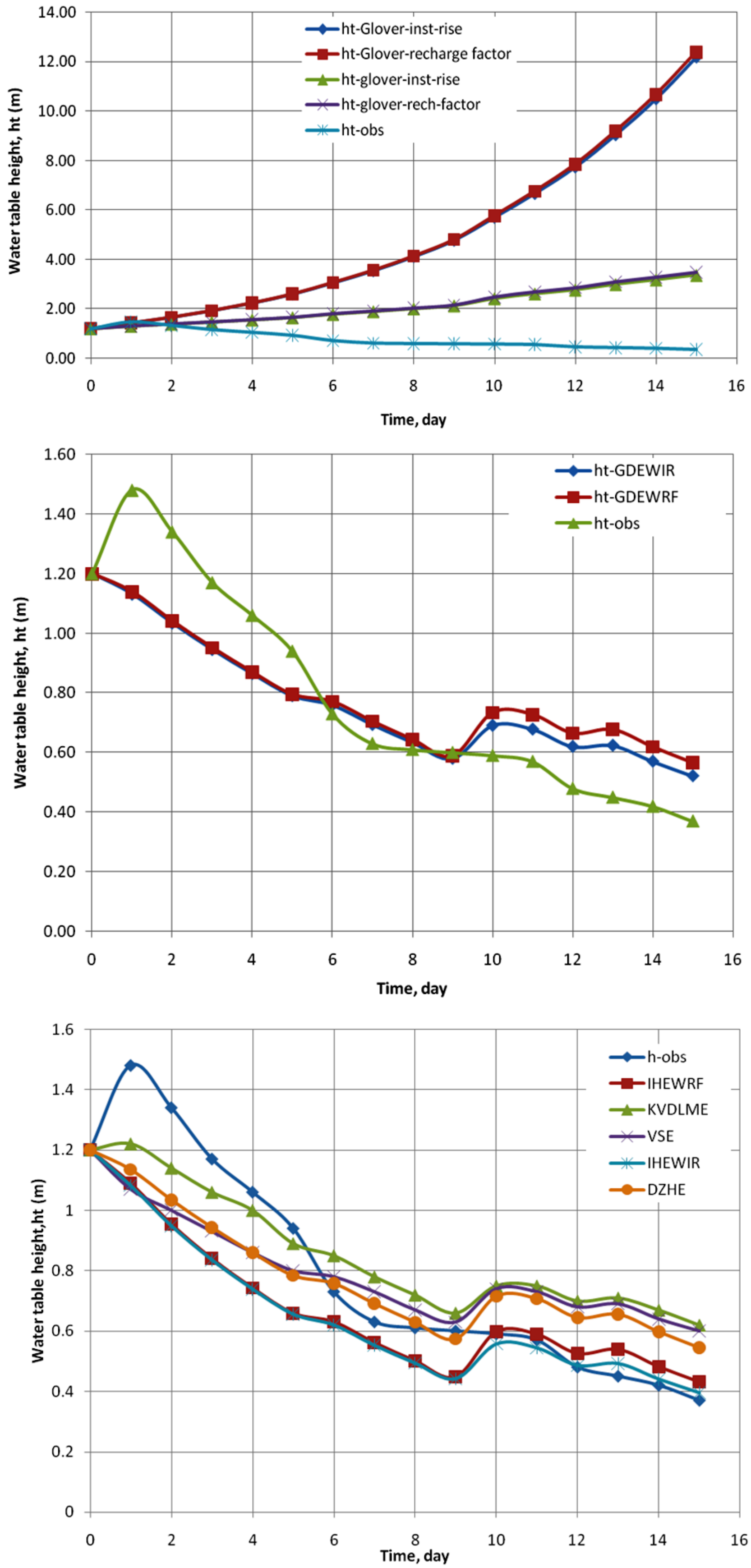

Fig. 1 Water table fluctuation in 50 m drain spacing plot

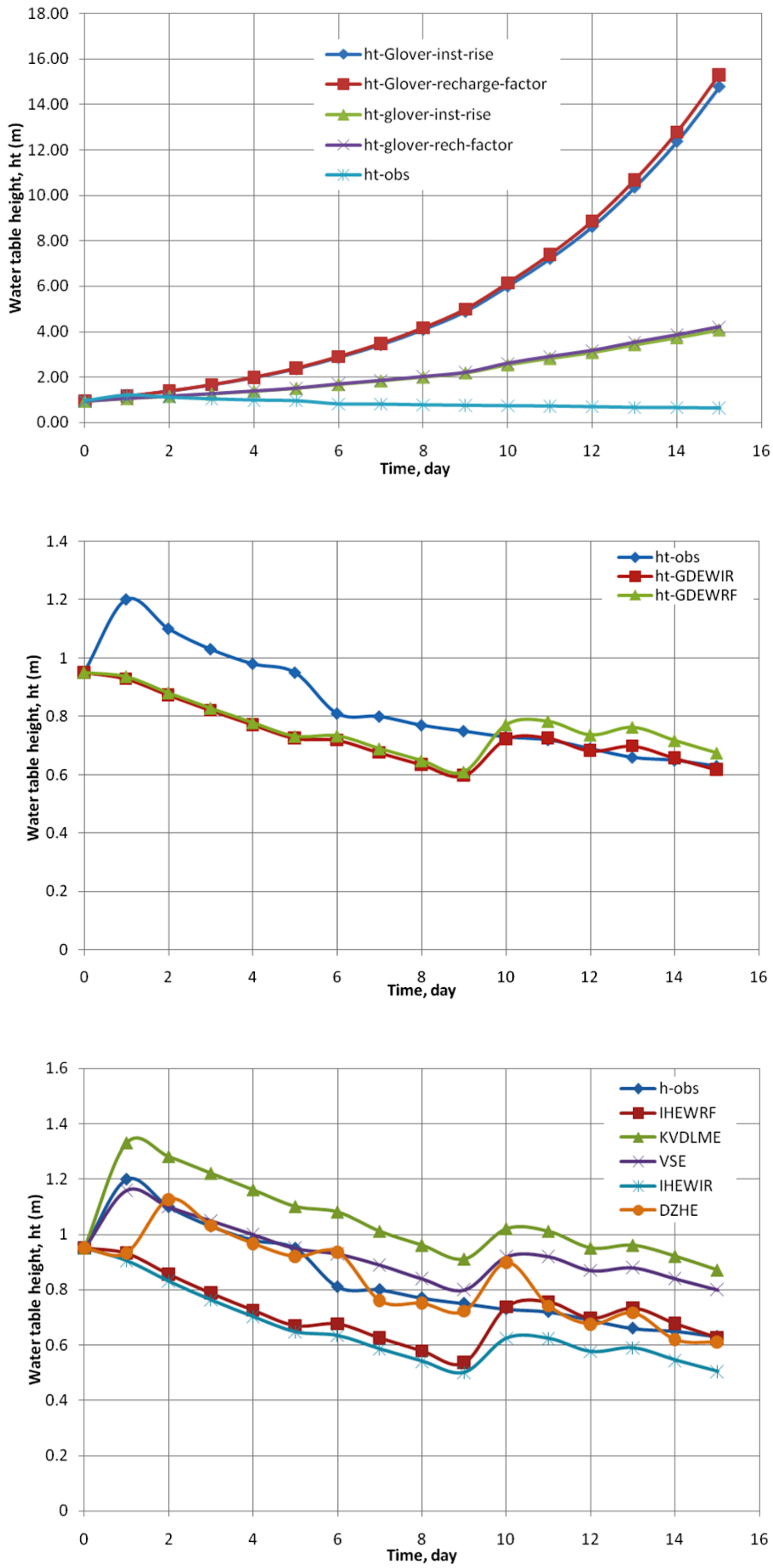

Fig. 2 Water table fluctuation in 75 m drain spacing plot

Table 2. Percentage deviation of predicted water table height by various equations

Time	50 m drain spacing					75 m drain spacing				
	IHEWRF	IHEWIR	KVDLM	VSE	DZHE	IHEWRF	IHEWIR	KVDLM	VSE	DZHE
1	39.40	26.89	17.57	27.70	22.97	22.45	24.61	-10.83	3.33	21.67
2	40.66	29.21	14.93	25.37	22.39	22.30	24.57	-16.36	0.00	20.00
3	39.53	28.58	9.40	20.51	18.80	23.64	25.98	-18.45	-1.94	19.42
4	40.42	30.29	5.66	18.87	17.92	26.02	28.39	-18.37	-2.04	20.41
5	39.87	30.23	5.32	14.89	14.89	29.55	31.90	-15.79	0.00	23.16
6	23.26	15.06	-16.44	-6.85	-5.48	16.59	21.78	-33.33	-14.81	9.88
7	20.21	12.28	-23.81	-15.87	-12.70	21.94	26.87	-26.25	-11.25	13.75
8	25.90	19.06	-18.03	-9.84	-6.56	24.93	29.75	-24.68	-9.09	15.58
9	32.13	26.32	-10.00	-5.00	1.67	28.58	33.24	-21.33	-6.67	18.67
10	0.01	5.59	-27.12	-25.42	-23.73	-1.00	14.47	-39.73	-26.03	-5.48
11	-4.77	4.55	-31.58	-28.07	-28.07	-4.81	13.44	-40.28	-27.78	-8.33
12	-11.49	-1.30	-45.83	-41.67	-39.58	-0.90	16.57	-37.68	-26.09	-7.25
13	-25.10	-9.42	-57.78	-53.33	-51.11	-11.04	10.71	-45.45	-33.33	-15.15
14	-20.27	-4.97	-59.52	-52.38	-47.62	-4.08	16.19	-41.54	-29.23	-10.77
15	-22.75	-6.89	-67.57	-62.16	-54.05	0.74	19.97	-38.10	-26.98	-6.35
MD %	25.72	16.71	27.38	27.20	24.50	15.90	22.56	-28.54	-14.57	14.39
RMSD	0.2083	0.2110	0.1663	0.2020	0.1769	0.1779	0.2070	0.2187	0.1226	0.0911

IHEWRF-Integrated Hooghoudt Equation With Instantaneous Rise, IHEWIR=Integrated Hooghoudt Equation With Rise Factor, KVDLM=Kraijenhoff van de Leur- Maasland, van Schilfgaarde Equation, DZHE=de Zeeuw Hellinga Equation

order of performance of these equations based on mean per cent deviations and RMSD are KVDLM, GDEWIR, DZHE, GDEWRF, VSE, IHEWRF and IHEWIR.

75 m Drain spacing plot

From Fig. 2 it can be seen that GEWIR, GEWRF, DEWIR and DEWRF could not predict water table trend and indicated only rising trend. Their respective per cent deviations ranged from 2.59 to -2244.34%, 2.35 to -2332.55%, -6.17 to -546.69% and -6.97 to -568.20% and corresponding mean per cent deviations of -680.45%, -702.87%,-204.30% and -211.50%, respectively. RMSD of GEWIR, GEWRF, DEWIR and DEWRF were calculated as 6.3634, 6.5872, 1.7998 and 1.8670, respectively. GDEWIR and GDEWRF predicted water table heights which matched well with the values observed in the fields. The per cent deviations of predicted water table heights by GDEWIR and GDEWRF over observed values ranged from -0.77 to 23.65% and -5.64 to 22.99% with corresponding mean percent deviations of 12.35% and 14.46% and RMSD of 0.1486 and 0.1472, respectively.

Fig. 2 shows that IHEWRF, IHEWIR, KVDLM, VSE and DZHE predicted water tables quite well in 75 m drained field. The percent deviations of predicted water table heights by IHEWRF, IHEWIR, KVDLM, VSE and DZHE over observed values ranged from -0.90 to 29.55%, 10.71 to 33.24%,-0.83 to -45.45%, 0.00 to -33.33% and -5.48 to 23.16% with correspond mean per cent deviations of 15.90%, 22.56%, -28.54%, -14.57% and 14.39% and RMSD of 0.1779, 0.2070, 0.2187, 0.12026 and 0.0911, respectively. The DZHE gave the lowest mean per cent deviations and RMSD values. The equations with rise factor performed well with their order of performance as DZHE, VSE, IHEWRF, IHEWIR and KVDLM. GDEWIR and GDEWRF also performed well with closer values of water table height with each other. The GDEWRF and GDEWIR performed better than IHEWRF, IHEWIR and KVDLM. The overall order of performance of these equations based on mean per cent deviations and RMSD are DZHE, VSE, GDEWRF, GDEWIR, IHEWRF, GDEWIR and KVDLM. Glover (1954) equation which assumes a flat water table and Dumm (1960) as a forth degree parabola of initial water table heights are not suitable for predicting water table fluctuations in response to rain with rise factor or instantaneous recharge. Glover (1954) equation has shape factor as 1.27 (4/π) and Dumm (1960) has shape factor 1.16. Integrated Hooghoudt Equation has a variable shape factor $\left(\frac{2d_e + h_t}{2d_e + h_o}\right)$ and unit shape factor can be used for predicting water table fluctuations.

GDEWRF and DZHE are the same equation except for difference in shape factor. In DZHE has a shape factor of 0.80 and GDEWRF has shape factor 0.78. IHEWRF was ranked at sixth in 50 m drain spacing plot and fifth in 75 m drain spacing plot. Peaks of water table height after rainfall are quite noticeable by all the water table predicting equations, but are not distinctive in the case of the observed water table heights. However, the effect of rainfall is visible as the slope of the curve is flatter in the later phase of curve. Water table heights were recorded in the morning and the evening. The peak might have resulted in between of these two measurements. Intermittent low intensity rainfall may not result in a single peak while substitution accumulated rainfall within 24 hour will result in a peak. All the available models ignores flow above drain level or average thickness of aquifer (equivalent depth of impervious layer below drain axis, d_e + half the initial water table height above drain axis, 0.5 h_o) can considered for averaging the result. The drainage plots having drain spacing of 50 m and 75 m have corresponding depths of impervious layer below drain axis as 1.2 m while depth of drain line is 1.75 m below ground surface. The initial water table height was only 1.20 m for 50 m drain spacing plot and 0.95 m for 75 m drain spacing plot, hence flow above drain level was not very high. The Integrated Hooghoudt Equation which considers flow below and above the drain level was transformed to predict water table fluctuations in response to constant recharge or rainfall based on sound hypothesis has added advantage for its field applicability. The approach can be applied for using any transient drain spacing formula for simulating water table fluctuations in response to constant recharge.

Conclusions

An approach was developed for predicting rise of water table between parallel subsurface drains and predicting water table fluctuations in response to constant recharge or rainfall which could be successfully used for transforming any transient drain spacing equations for predicting water table fluctuations between the drains. The Integrated Hooghoudt transient solution which takes radial flow above drain level into account was transformed into a form to predict water table fluctuation. The equation was tested in 50 m and 75 m drain spacing plots. The classical transient drain spacing formulas of Glover (1954) and Dumm (1960) were also transformed for predicting and comparing water table fluctuations in response to rain. de Zeeuw and Hellinga (1958), Kranjenhoff van de Leur (1958) and Maasland (1959), van Schilfgaarde (1965) were compared with Transformed Integrated Hooghoudt Equation with recharge factor and instantaneous recharge. The order of performance in descending order were Kranjenhof van de Leur-Maasland, Glover-Dumm Equation with instantaneous rise, de Zeeuw-Hellinga Equation, Glover-Dumm Equation with recharge factor, van Schilfgaarde Equation, Integrated Hoohgoudt Equation with recharge factor and Integrated Hooghoudt Equation with instantaneous recharge with their corresponding RMSDs as 0.1663, 0.1729, 0.1769, 0.1879, 0.2020, 0.2083 and 0.2110 in 50 m drained plot. Similarly in 75 m drained plot order of performance were observed as de Zeeuw-Hellinga Equation, van Schilfgaarde Equation, Glover-Dumm Equation with recharge factor, Glover-Dumm Equation with instantaneous rise, Integrated Hoohgoudt Equation with recharge factor, Glover-Dumm Equation with instantaneous rise and Kranjenhof van de Leur-Maasland with corresponding RMSDs of 0.0911, 0.1226, 0.1472, 0.1486, 0.1779, 0.2070 and 0.2187. Integrated Hooghoudt Equation with recharge factor which considers flow above drain level is recommended for further field applications.

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Impact of Soil Texture on Different Organic Carbon Pools in Sirsa District of Western Haryana, India

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Abstract

Soil organic carbon (SOC) pools are important in maintaining soil productivity and influencing the CO_2 loading into the atmosphere. Different soil textural classes of Sirsa district, Haryana were compared for SOC and its fractions *viz*; active (very labile, VLSOC; labile, LSOC) and passive (less labile, LLSOC; non-labile, NLSOC) pools. Maximum OC (0.66%) and TOC (0.78%) was observed in clay loam texture compared to loamy sand, sandy loam, loam and clay loam texture. Similarly, highest VLSOC (0.47%) and LSOC (0.11%) pools were recorded in sandy soils whereas highest LLSOC (0.12%) and NLSOC (0.18%) pools were recorded in clay loam soils compared to other textures. Highest RI_1 (0.50) and RI_2 (0.23) were found in clay loam soils whereas highest carbon lability index (CLI) was found in sandy (3.96) soils. The texture of soil significantly impacted SOC and its associated pools. VLSOC had significant positive correlation with LSOC ($p \leq 0.05$), while LSOC showed significant positive correlation with VLSOC ($p \leq 0.01$). Similarly, LLSOC had significant positive correlation with NLSOC reciprocated by NLSOC exhibiting a significant positive correlation with LLSOC ($p \leq 0.01$). Therefore, correlation amongst the pools of C showed that most of the pools were significantly correlated with each other.

Key words: Soil organic carbon pools, Soil texture, Recalcitrant index, Carbon lability index

Introduction

Soil organic carbon (SOC) plays a crucial role in sustaining soil resilience, which affects ecosystem services and climate change (Bhattacharyya *et al*., 2009; Wong *et al*., 2010). Since soil contains most of the terrestrial carbon, any effort to increase its concentration is likely to enhance the biological properties of soil. Due to its profound effects on climate change and potential benefits for crop productivity, understanding the dynamics of organic carbon accumulation in agricultural soils is becoming more and more important. By enhancing soil fertility and productivity, good agricultural practices can transform such soil into a net sink for carbon, reducing the amount of CO_2 in the atmosphere (Lal, 2004). The amount of soil organic carbon (SOC) at a given time represents the long-term equilibrium between the addition of organic carbon from various sources and its depletion via various pathways. Naturally, it (SOC) varies with land usage, soil type, soil texture and climate zone (Swarup *et al.,* 2000). Large-scale intensive cropping causes long-term balance disruption due to large-scale addition of carbon to the soil through crop residues. This carbon influx either results in a net build-up or depletion of SOC stock, or it exposes more and more of the C to oxidative losses due to continued cultivation (Kong *et al*., 2005). This stock of SOC is made up of stable, passive, recalcitrant pools with different residence times, as well as labile or actively cycling pools. Among these, the portion of SOC with the fastest turnover rates is known as the labile carbon pool (LCP). The movement of CO_2 from soils into the atmosphere is caused by oxidation of this particular carbon pool. LCP is crucial because it feeds the soil food web, which in turn affects nutrient cycling, critical for maintaining soil productivity and quality (Chan *et al*., 2001; Mandal, 2005; Mandal *et al*., 2007). The majority of current techniques for calculating SOC were created to optimize C oxidation and recovery (Walkley and Black, 1934; Nelson and Sommers, 1982). The characterization of SOC resulting from various soil management practices, such as cropping systems and the application of organic and inorganic sources of nutrients, may benefit from the use of techniques that can preferentially extract the more labile pools.

Carbon pool that is not readily available for microorganisms to access and require more time to decompose is the recalcitrant carbon pool (Lal, 2004). Recalcitrant carbon pools are highly variable in terms of their chemical composition and state of decomposition, and they are important for the health and function of soil (Stevenson, 1994). Humic compounds comprise 60%–80% of the total SOC and have the highest concentration of humin (de Almeida *et al*., 2014). Due to its higher fraction of aromatic functions and bonds to mineral components, humin has the strongest resilience against microbial degradation and is found in the highest concentration in soil (Lal, 2004). Thus, in tropical environments, both labile and recalcitrant carbon pools can provide information about the soil's past utilization and the best management practices for increasing carbon stocks (Basak *et al*., 2021)

The amount and distribution of different SOC pools depends on several factors like temperature, moisture, land use management, soil texture, agronomic practices etc. Among these factors, soil texture is a critical determinant of SOC pools within specific climatic conditions as protection of soil organic carbon from microbial breakdown strongly relies on soil texture. Hereunder, our objective was to determine how the quality and concentration of SOC pools varied among the various soil texture in Sirsa district of Haryana, India.

Material and Methods

Description of study area

Sirsa is a north-western district of Haryana state, located between latitudes 29°14' to 30°0' N and longitudes 74°29' to 75°18' E. Out of total geographical area of 4276 km^2, 4050 km^2 area is under cultivation.

The seven blocks of the district—Nathusari Choupta, Rania, Sirsa, Baragudha, Odhan, Ellenabad, and Dabwali—are included in the study area (Fig. 1). The Sirsa district has a hot, dry tropical climate. In May and June, the mean

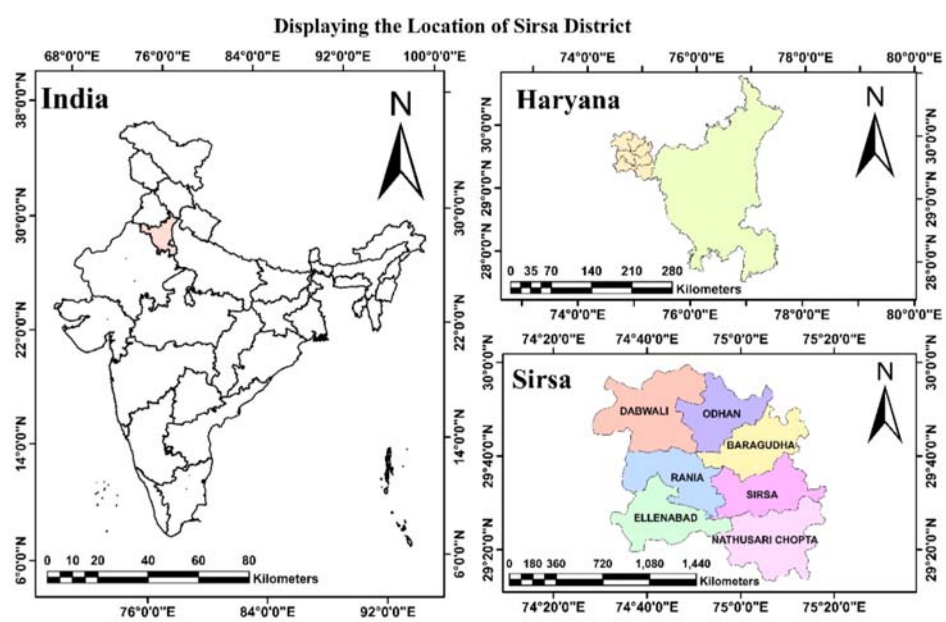

Fig. 1 Location map of study area

maximum temperature is 41.1°C, while in January, the mean lowest temperature is 5.1°C. The district receives an average rainfall of 318 mm, including 253 mm of monsoon rainfall. Major cropping systems in the area are cotton- wheat, rice – wheat, groundnut – mustard, pearl millet – wheat, cluster bean – mustard, green gram – wheat and guava.

Soil sampling and laboratory analysis

Total 350 soil samples (up to 0-15 cm soil depth) were collected randomly from seven blocks of Sirsa district. Out of these samples 15 samples from each texture (determined by feel method) except clay loam (7) sorted for further analysis. All the samples were brought to the lab, air-dried, manually crumbled to remove the root materials, and then passed through 0.5 mm sieve. The pH and electrical conductivity of soils were measured using 1:2 soil-water suspension with standard procedures as described by Jackson (1973). Organic carbon (OC) was determined using the rapid titration method (Walkley and Black, 1934). Total organic carbon(TOC) content of soil samples was evaluated by wet oxidation method using a 1N $K_2Cr_2O_7$ (potassium dichromate) solution followed by one hour of heating at 150°C (Snyder and Trofymow, 1984).

Oxidizable pools of SOC

The oxidisable organic C content of the soil was determined by modified Walkley and Black method as described by Chan *et al*. (2001) (Table 1). The total organic C (TOC) might be divided into the following four pools based on decreasing order of oxidizability.

The VLSOC and LSOC together constitute the active pool [Active pool = Σ(VLSOC + LSOC)]; while LLSOC and NLSOC collectively represents the passive pool [Passive pool = Σ(LLSOC + NLSOC)] of organic C in soils (Chan *et al*., 2001).

Carbon Lability Index (CLI)

Equation (1) provided by Majumder et al. (2007) was used to calculate the carbon lability index (CLI) of SOC based on the relative oxidizability of the CVL, CL, and CLL pools of SOC.

$$\text{Carbon Lability Index (CLI)} = \frac{VLSOC}{TOC} \times 3 + \frac{LSOC}{TOC} \times 2 + \frac{LLSOC}{TOC} \times 1 \quad (1)$$

Recalcitrant Index (RI)

The recalcitrant index (RI) of SOC was computed to assess effect of soil texture on the stability of organic C in the soil by using Equations (2) and (3) given by Datta *et al*. (2018) as:

$$RI_1 = \frac{CLL+CNL}{CVL+CL} \quad (2)$$

$$RI_2 = \frac{CNL}{TOC} \quad (3)$$

Between the two indices, RI_1 indicates the relative amount of labile and non-labile SOC pools; on the other hand, RI_2 represents the percentage of non-labile. To evaluate the soil organic C stability, place the SOC pool over the TOC. RI mean was determined by averaging RI_1 and RI_2 for the corresponding soil texture (Basak *et al*., 2021).

Descriptive statistics of the analysed soil data viz., minimum, maximum , mean value and standard deviation were determined using SPSS software (29.0.1.0). Correlation matrix among different parameters was prepared using OPSTAT software of Haryana Agricultural University (Sheoran *et al*., 1998).

Table 1. Description of soil organic carbon pools

S.No.	Carbon Pools	Description
1.	VLSOC, Very Labile Soil Organic Carbon	Organic C oxidizable under 12.0 N H_2SO_4
2.	LSOC, Labile Soil Organic Carbon	Difference in SOC extracted between 18.0 N and 12.0 N H_2SO_4
3.	LLSOC, Less Labile Soil Organic Carbon	Difference in SOC extracted between 24.0 N and 18.0 N H_2SO_4 (the 24.0 N H_2SO_4 is equivalent to the standard Walkley and Black method)
4.	NLSOC, Non-Labile Soil Organic Carbon	Residual organic C after reaction with 24.0 N H_2SO_4 when compared with TOC

Table 2. Descriptive statistics of physico-chemical properties of soil under different texture in Sirsa district

Soil Texture	pH				EC (dS m^{-1})				OC (WBC) (%)				TOC (%)			
	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD
Sandy	8.70	7.40	8.27	0.22	0.75	0.16	0.32	0.18	0.51	0.30	0.38	0.06	0.53	0.35	0.43	0.05
Loamy Sand	8.74	7.50	8.35	0.17	0.82	0.12	0.37	0.22	0.55	0.32	0.45	0.06	0.66	0.41	0.55	0.08
Sandy Loam	8.71	7.50	8.38	0.18	0.69	0.15	0.35	0.23	0.61	0.40	0.52	0.06	0.72	0.49	0.63	0.07
Loamy	8.75	7.55	8.47	0.18	0.71	0.20	0.38	0.16	0.77	0.50	0.62	0.08	0.88	0.62	0.76	0.08
Clay Loam	8.80	7.62	8.48	0.15	0.80	0.34	0.41	0.19	0.75	0.55	0.66	0.07	0.90	0.72	0.78	0.05

EC: Electrical Conductivity; OC: Organic Carbon (Walkley and Black Carbon); TOC: Total Organic Carbon; SD: Standard Deviation

Results and Discussion

Soil pH and electrical conductivity

It was observed that soil pH varied significantly under different soil texture. Average soil pH of sandy, loamy sand, loam, sandy loam, clay loam texture was 8.27, 8.35, 8.38, 8.47 and 8.48, respectively (Table 2). Among different soil texture, the lowest soil pH was observed in sandy (8.27) soils whereas the highest soil pH was observed in clay loam (8.80) soils. This might be due to more leaching of basic ions in sandy soils compared to clay loam soils (Ulrich and Sumner, 2012).

Electrical conductivity exhibited variability under different soil texture. Average EC of sandy, loamy sand, loam, sandy loam, clay loam texture was 0.32, 0.37, 0.35, 0.38, and 0.41 dS m^{-1}, respectively (Table 2). The highest EC was recorded in clay loam (0.80 dS m^{-1}) texture while lowest EC was recorded in sandy (0.32 dS m^{-1}) soils. This was probably due to higher surface area in clay loam soils than sandy soils, that facilates more particle-to-particle contact, coupled with higher moisture retention capacity, making them more conductive (Rhoades and Corwin, 1990).

Soil organic carbon (Walkley and Black carbon) and total organic carbon

Maximum SOC (0.66%) and TOC (0.78%) content was recorded in clay loam soils while minimum value of SOC (0.38%) and TOC (0.43%) were recorded in sandy soils (Table 2). This might be due to less aeration in clay loam soil texture, which results in lower rate of oxidation of organic matter and consequently increase the carbon storage (SOC and TOC) (Antil *et al.*, 2016; Gora, 2013).

Soil organic carbon fractions

Oxidizable fractions of organic carbon significantly varied under different soil texture. Maximum VLSOC (0.47%) and LSOC (0.11%) fractions were observed in sandy texture while minimum VLSOC (0.18) and LSOC (0.05%) fractions was observed in clay loam soils (Table 3). It might be attributed to more aeration in sandy soils which promotes the conversion of non-labile or recalcitrant pool in very labile and labile pools by decomposition process (Gillis and Price, 2011; Marschner *et al.*, 2008). Similarly, maximum LLSOC (0.12%) and NLSOC (0.18%) pools were recorded in clay loam soils whereas minimum

Table 3. Descriptive statistics of oxidizable organic carbon pools under different soil texture in Sirsa district

Soil Texture	VLSOC (%)				LSOC (%)				LLSOC (%)				NLSOC (%)			
	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD
Sand	0.56	0.35	0.47	0.06	0.09	0.01	0.11	0.03	0.13	0.02	0.06	0.03	0.11	0.01	0.05	0.03
Loamy Sand	0.55	0.37	0.44	0.05	0.11	0.01	0.10	0.03	0.27	0.02	0.08	0.07	0.15	0.03	0.09	0.03
Sandy Loam	0.53	0.31	0.38	0.06	0.24	0.01	0.08	0.05	0.13	0.02	0.09	0.03	0.19	0.01	0.11	0.05
Loamy	0.33	0.23	0.28	0.03	0.24	0.03	0.05	0.05	0.25	0.02	0.10	0.06	0.19	0.11	0.15	0.03
Clay Loam	0.23	0.14	0.18	0.03	0.28	0.05	0.05	0.06	0.21	0.02	0.12	0.06	0.25	0.09	0.18	0.04

VLSOC: Very Labile Soil Organic Carbon; LSOC: Labile Soil Organic Carbon; LLSOC: Less Labile Soil Organic Carbon; NLSOC: Non-Labile Soil Organic Carbon

LLSOC (0.06%) and NLSOC (0.05%) pools were recorded in sandy soils (Table 3). This might be due to less aeration in clay loam soils and consequently lower rate of decomposition of less labile and non-labile or recalcitrant carbon pools (Marschner *et al.*, 2008).

Recalcitrant index (RI) and carbon lability index (CLI)

Maximum RI_1 (0.50) and RI_2 (0.23) were observed in clay loam soils whereas minimum RI_1 (0.38) and RI_2 (0.11) were observed in sandy soils (Table 4). This might be due to higher amount of recalcitrant carbon and less labile carbon in clay loam soil (Basak *et al.*, 2021). Highest CLI (3.96) was recorded in sandy soils while minimum (0.91) was recorded in clay loam soils (Table 4). This might be attributed to higher amount of very labile, labile and less labile pools in sandy soils (Basak *et al.*, 2021).

Relationship among different organic carbon pools and soil properties

Correlation analyses revealed a significant correlation among majority of carbon pools (Table 5). Soil pH showed significant positive correlation with OC, TOC, LLSOC and NLSOC ($p \leq 0.01$). EC also displayed significant positive correlation with OC, TOC, LLSOC, NLSOC ($p \leq 0.05$). Similarly, TOC showed significant positive correlation with LLSOC and NLSOC ($p \leq 0.01$). VLSOC had significant positive correlation with LSOC ($p \leq 0.05$) and strong negatively correlation with LLSOC and NLSOC ($p \leq 0.01$). LSOC showed significant positive correlation with VLSOC ($p \leq 0.01$) whereas strong negative correlation with LLSOC and NLSOC ($p \leq 0.01$) was recorded. Similarly, LLSOC had significant positive correlation with NLSOC and NLSOC had significant positive correlation with LLSOC ($p \leq 0.01$).

Table 4. Descriptive statistics of recalcitrant index-1 (RI_1), recalcitrant index-2 (RI_2) and carbon lability index (CLI)

Soil Texture	RI_1				RI_2				CLI			
	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD
Sand	0.91	0.10	0.38	0.25	0.25	0.02	0.11	0.07	4.84	2.99	3.96	0.51
Loamy Sand	0.87	0.22	0.50	0.19	0.26	0.07	0.17	0.06	3.77	2.37	3.05	0.53
Sandy Loam	0.64	0.16	0.39	0.14	0.27	0.02	0.18	0.07	2.56	1.91	2.15	0.20
Loamy	0.70	0.25	0.47	0.13	0.23	0.11	0.18	0.04	1.72	1.11	1.40	0.18
Clay Loam	0.63	0.35	0.50	0.10	0.28	0.12	0.23	0.05	0.98	0.76	0.91	0.08

RI: Recalcitrant Index; CLI: Carbon Lability Index; SD: Standard Deviation

Table 5. Correlation among different soil properties and carbon fractions

Variable	pH	EC	OC (WBC)	TOC	VLSOC	LSOC	LLSOC	NLSOC	RI_1	RI_2	CLI
pH	1										
EC	0.901*	1									
OC (WBC)	0.990**	0.879*	1								
TOC	0.997**	0.878*	0.994**	1							
VLSOC	-0.937*	-0.877NS	-0.971**	-0.939*	1						
LSOC	-0.979**	-0.815NS	-0.989**	-0.984**	0.949*	1					
LLSOC	0.959**	0.931*	0.973**	0.962**	-0.963**	-0.927*	1				
NLSOC	0.985**	0.925*	0.993**	0.985**	-0.975**	-0.965**	0.991**	1			
RI_1	0.636NS	0.866NS	0.561NS	0.582NS	-0.554NS	-0.492NS	0.626NS	0.632NS	1		
RI_2	0.889*	0.935*	0.890*	0.890*	-0.870NS	-0.813NS	0.967**	0.930*	0.679NS	1	
CLI	-0.929*	-0.922*	-0.883*	-0.919*	0.791NS	0.837NS	-0.905*	-0.904*	-0.759NS	-0.821*	1

*Significant at the 0.05 level (2-tailed) and **Significant at the 0.01 level (2-tailed)

EC: Electrical Conductivity; OC (WBC): Organic Carbon (Walkley and Black Carbon); TOC: Total Organic Carbon; VLSOC: Very Labile Soil Organic Carbon; LSOC: Labile Soil Organic Carbon; LLSOC: Less Labile Soil Organic Carbon; NLSOC: Non-Labile Soil Organic Carbon; RI: Recalcitrant Index; CLI: Carbon Lability Index

Conclusions

This study underscores the role of soil texture in shaping SOC dynamics. A consistent trend was recorded where higher clay and silt content increased SOC levels under similar climatic conditions. Highest SOC and TOC was recorded in clay loam soils. Similarly, highest very labile and labile fraction were recorded in sandy soils whereas less labile and non-labile or recalcitrant carbon were recorded in clay loam soils. Furthermore, indices like RI1, RI2, and CLI exhibit texture-specific patterns, affirming the profound effect of soil texture on organic carbon variations. The interrelationships observed among different carbon pools through correlation analyses further emphasize the interconnected nature of these fractions within soil systems, highlighting the complex dynamics governed by soil texture.

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Effect of Gypsum and Zinc on Soil Properties under Sodic Irrigation in South Western Haryana

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Abstract

An experiment was conducted at farmers' field in Mahendergarh District, to study the effect of gypsum and zinc on soil properties under sodic irrigation. The experiment was laid out in a randomized block design with three replications and treatments comprised four levels of gypsum (G_0, G_{50}, G_{75} and G_{100}) and four levels of zinc (0, 5, 10 and 25 kg Zn ha^{-1}). The mean soil pHs (9.11) in control is significantly decreased with increase in gypsum levels up to G_{100} (8.56) whereas zinc application did not significantly influence the soil pH. Significantly higher soil $EC_{1:2}$ (0.88 dS m^{-1}) was recorded under G_{100} treatment. While the lowest soil EC (0.43) was recorded under control (G_0). Significantly higher organic carbon (0.39%) was observed in G_{100} treatment over the G_0 (0.31%). Exchange sodium percent (ESP) was significantly decreased with the increase in gypsum level. Significantly decrease in ESP (33.0%) was recorded under G_{100} treatment over control G_0 (43.3). Whereas zinc application did not significantly influence the soil pH and EC, organic carbon and ESP. The available N, P, K, Zn and Ca in soil is significantly increased with the application of gypsum and zinc over control. The interactive effect of gypsum and zinc in this present investigation was found non-significant.

Key words: Gypsum, Zinc, Sodic water, Soil properties

Introduction

In India, wheat (*Triticum aestivum*) is a widely grown crop and a significant source of staple foods. It is considered the "king of cereals" and is the second most popular food grain after rice. It belongs to the family Poaceae, contains approximately 8-10% protein, 78% carbohydrate, 2% fat and minerals, and some amounts of vitamins too (Kumar *et al.*, 2011) and provides a balanced food to millions of people each day. Salt-affected soils are an important ecological entity in India and around 6.7 M ha area is affected in different climatic regions (NRSA,1996). Out of this, 3.77 M ha area is in sodic soil and 2.95 M ha area under saline soil and degradation resulting from sodicity is a major obstacle for optimal utilization of land resources. The 3.78 mha soils in India including 0.18 mha soils in Haryana are affected by sodicity. Out of total cultivated area of 3.62 mha, 1.24 mha is canal irrigated, 1.65 mha is irrigated by tube wells which often contain water of dubious quality, 0.73 mha is non irrigated area. In the state, 37% of water is of good quality, 8% normal and 55% is of poor-quality of which 11% is saline, 18% is alkali and 26% as saline-alkali (Manchanda, 1976). Groundwater is either the major or the only source of irrigation, in many parts of the arid and semi-arid regions. Most of the groundwater is high residual sodium carbonate (RSC) which is a serious problem. Amongst the various categories of poor-quality water, alkali (sodic) water has greater irrigation potential by virtue of these low salinity and in amenability for reclamation especially in semi-arid and arid region of north west India where their occurrence in groundwater is around 30-54% (Minhas and Bajwa, 2001). Scarcity of good quality water in these regions often forces the farmers to use available poor-quality water for irrigation. Sodic waters are characterized by their low EC (<4 dS m^{-1}), high sodium adsorption ratio (SAR) and high RSC which constitute most important source of supplemental irrigation provided they are used carefully. Such waters are found in vast areas of Rewari, Jhajjar, Bhiwani, Mahendergarh, Gurgaon, Sirsa, Kaithal, Hisar and Fatehabad districts of Haryana. By combining the necessary amount of gypsum with the sodic water, it is possible to profitably use this resource. The amount of gypsum needed will largely depend on the residual sodium carbonate (RSC) in the sodic water, as well as the type of soil to be irrigated, the crops to be grown, and the amount of annual precipitation received. With the intensive cropping of high yielding varieties of rice and wheat in the state, deficiency of zinc initially, and subsequently deficiency of iron in rice and manganese in wheat emerged as threats to sustaining high level of food grain production (Singh, 2009). Therefore, the study was carried out on the requirement of different level of gypsum and zinc in wheat crop irrigated with sodic water. Due to its affordability, gypsum is the most often utilized amendment for reclaiming sodic soil. Gypsum added to a sodic soil can initiate permeability increases due to both electrolyte concentration and cation exchange effects (Loveday, 1976). The relative significance of the two effects is of interest for several reasons. If the electrolyte effect is significantly great to prevent dispersion and swelling of soil clays, the surface application of gypsum may be worthwhile. Among various micronutrients, zinc is one of the important elements for plant growth and it activates many enzymes which are involved in metabolic processes and biochemical pathways. It acts as a functional, structural or regulatory co-factor for many enzymes and has key role in DNA transcription. Nijra and Nabwami (2015) reported that it influences the formation of chlorophyll and auxins which resulted in formation of the growth promoting compounds. Zinc plays an outstanding role in synthesis of chlorophyll and protein and also regulates inter absorption. Moreover, it is also concerned with carbohydrate metabolism and activation of various enzymes. Zinc application recommendation in soils depends upon crop, soil type, pH and other nutrient status. Zinc deficiency in sodic soils is quite common due to excessive sodium ions, high pH, carbonate and bicarbonate ions. Zinc also helps in inducing alkalinity tolerance in crops by enhancing crops efficiently to utilize K and Ca. Thus, it reduces the Na/K and Na/Ca ratio in plant tissues to mitigate the adverse effect of alkalinity in crop which is an important aspect (Mishra, 2001). So, taking into consideration, the importance of gypsum and zinc in wheat crop production under sodic water irrigation, the research experiment entitled, "Effect of gypsum and zinc on soil properties and wheat (*Triticum aestivum*) yield under sodic water irrigation" was undertaken at farmer field in Mahendergarh district of Haryana during *Rabi* 2021.

Material and Methods

A field experiment was conducted during *rabi* seasons of the years 2021 and 2022 at farmers' field in Mahendergarh district of south-west Haryana located at latitude 28.19° N, longitude 76.59° E and 266 m above mean sea level. The climate of Mahendergarh is characterized as dry sub-humid with normal rainfall of about 577 mm. The south-west monsoon (July-September) with an average contribution of 83% of total annual rainfall is the main rainfall season at Mahendergarh. The crops in the experimental site for the previous two years were as in 2019-20 i.e., fallow-mustard in 2020-21 and pearl millet-wheat 2021-22. The pearl millet-experimental crop was grown during *kharif* (monsoon) and wheat during *rabi* (winter) season. The experiment was laid out in randomised block design (RBD) under 16 treatments with three replications. Wheat was grown under sodic water irrigation having RSC (5.2 meq l^{-1}) with four levels of gypsum and Zn were applied. The treatment without gypsum (G0) was control and G_{50}, G_{75} and G_{100} (of required dose) were applied for neutralization of RSC and Zn_0, Zn_5, Zn_{10} and Zn_{25} kg Zn was applied per hectare with recommended dose of N, P and K. Soil pH was then determined using pH meter consisting of glass electrode in 1:2 (soil: water) suspension at room temperature 25°*C (*Jackson, 1973). Electrical conductivity was determined using a conductivity meter in 1:2 (soil: water) suspension at room temperature 25°C. The oxidizable soil organic carbon content was determined by Walkley and Black (1934) method. Exchangeable sodium was extracted by neutral normal ammonium acetate (pH 7.0) and estimation was done by using flame photometer.

Available Nitrogen (N) was determined by alkaline permanganate method (Subbaiah and Asija, 1956). Available P content was determined by extracting the soil samples using 0.5N $NaHCO_3$ (pH 8.5) and analyzed by spectrophotometer as described by Olsen *et al.* (1954). Available K was extracted in neutral normal ammonium acetate (pH 7.0) and was determined on a flame photometer as described by Jackson (1973). For the determination of Ca individually, the Versenate titration method (Cheng and Bray, 1951) was used in which EDTA, disodium salt, solution was used to chelate them. Available sulphur was extracted with a solution of calcium chloride and was determined on a spectro-photometer at 420 nm wavelength by using blue filter, as described by Chesnin and Yien (1950). Available-Zn content in soil samples was determined by DTPA methods of Lindsay and Norvell (1978) using Atomic Absorption Spectrophotometer (Varian- Spectra AA-240S). Initially, soil $pH_{1:2}$ was in alkaline (9.15) range, $EC_{1:2}$ 0.35 dS m^{-1}, organic carbon 0.30%, ESP 45.3, available nitrogen, available P, available K were 110.3, 10.1, 165.5 kg ha^{-1}, respectively. Soil contained available sulphur, calcium and zinc 38.60, 46.8 kg ha^{-1} and 0.65 ppm, respectively.

Results and Discussion

Effect of gypsum and zinc application on soil pH and EC

The soil pH is significantly decreased with increase in gypsum levels up to G_{100} treatment. The soil pH was greater at G_0 treatment with 9.11 and the minimum soil pH was 8.56 in G_{100}. The similar values of soil pH were recorded in G_{75} and G_{50} with the value of 8.81 and 8.82, respectively (Table 1). The electrical conductivity of soil increased significantly with the increased doses of gypsum application. The lower values of EC was observed at G_0 (0.43 dS m^{-1}) and the greater values of EC was observed at G_{100} (0.88 dS m^{-1}). The application of zinc did not significantly influence the soil pH and EC and interactive effect of gypsum and was non-significant (Table 1). Sodic irrigation with high residual alkalinity is attributed to the precipitation of calcite in presence of high concentration of carbonates and bicarbonates and build-up of Na in the soil and showed a higher value in soil pH in control. The lower pH under G_{100}, G_{75} and G_{50} as compared to the G_0 may be due to supply of soluble Ca^{2+} which precipitates as $CaCO_3$ and $CaHCO_3$ also reported by Vanessa *et al*. (2008). It may be due to replacement of exchangeable Na^+ during Na^+-Ca^{2+} exchange and subsequent leaching. These results are in agreement with Singh and Singh (2014), Monika (2012), and Sharma *et al*. (2014). The higher EC under G_{100} as compared to G_0 could be simply because of the sustained addition of Na in the irrigation water, Na and Ca released from the soil exchange complex and salt released due to gypsum dissolution. Similar results were reported by Sekhonand Bajwa (1993) and Kaur *et al*. (2008).

Organic carbon and exchangeable sodium percent

Soil organic was significantly increased with application of gypsum whereas, soil ESP was significantly decreased with the application of

Table 1. Effect of Gypsum and Zinc application on soil pH and EC (dS m^{-1}) after harvest of wheat crop under sodic water irrigation

Gypsum Level	Level of Zn (kg ha^{-1})									
	pH					$EC_{(1:2)}$				
	Control	05	10	25	Mean	Control	05	10	25	Mean
G_0	9.16	9.12	9.10	9.05	9.11	0.33	0.50	0.40	0.47	0.43
G_{50}	8.91	8.85	8.80	8.74	8.82	0.50	0.63	0.70	0.73	0.64
G_{75}	8.79	8.84	8.81	8.77	8.81	0.63	0.73	0.77	0.73	0.72
G_{100}	8.65	8.56	8.53	8.50	8.56	0.81	0.87	0.90	0.93	0.88
Mean	8.88	8.84	8.81	8.77		0.57	0.68	0.69	0.72	
LSD ($p \le 0.05$)	Gypsum level (G)= 0.24 Level of Zinc (Zn)= NS G × Zn= NS					Gypsum level (G)= 0.13 Level of Zinc (Zn)= NS G × Zn= NS				

Table 2. Effect of gypsum and zinc application on soil OC (%) and ESP after harvest of wheatcrop under sodic water irrigation

Gypsum Level	Level of Zn ((kg ha^{-1})									
	OC (%)					ESP				
	Control	05	10	25	Mean	Control	05	10	25	Mean
G_0	0.30	0.31	0.31	0.31	0.31	45.30	41.77	42.40	43.57	43.26
G_{50}	0.33	0.33	0.34	0.35	0.34	39.63	36.20	37.60	37.03	37.61
G_{75}	0.35	0.35	0.36	0.36	0.35	38.57	37.53	36.53	35.60	37.06
G_{100}	0.37	0.38	0.39	0.41	0.39	34.43	32.77	33.33	31.53	33.02
Mean	0.34	0.34	0.35	0.36		39.48	37.07	37.47	36.93	
LSD ($p \leq 0.05$)	Gypsum Level (G)= 0.02 Level of Zinc (Zn)= NS G × Zn= NS					Gypsum Level (G)= 0.95 Level of Zinc (Zn)= NS G × Zn= NS				

gypsum from G_0 to G_{100} (Table 2). The percent increased in organic carbon 9.68, 12.90 and 25.80 percent was observed at $G_{50,}$ G_{75} and G_{100} treatments (p values < 0.05). The lower values of organic carbon (0.31%) were observed where no gypsum was applied (G_0) and the greater values was observed in G_{100} where 100% neutralization of RSC. The greater values of ESP (43.3) were observed in treatment G_0 and ESP sowing significantly decreased trend with the application of gypsum and minimum was observed in treatment G_{100} (33.02). The percent decreased in mean ESP 13.16, 14.3 and 23.7 were observed in treatment $G_{50,}$ G_{75} and G_{100} (Table 2).

The interactive effect between gypsum and zinc was found non-significant. Kaur *et al.* (2008) studied a long-term experiment on sodic water irrigation adversely affecting the soil organic carbon quality and increased the pH, electrical conductivity, sodium adsorption ratio, exchangeable sodium percentage (ESP) and bulk density and decreased final infiltration rate of the soil. Whereas, application of gypsum and organic amendments reversed these trends. Choudhary *et al.* (2011) reported that continuous irrigation with sodic water for 15 years without gypsum or organic material resulted in a gradual increase in soil pH, exchangeable sodium percentage (ESP), deterioration of soil physical properties, and decrease in yields of both rice and wheat. The application of gypsum and FYM increased the removal of Na in drainage water and decreased the pH, ESP, sodicity and improve the physical and chemical soil properties which influenced the available N, P and K in amended soil with gypsum and FYM. The decrease in ESP due to the removal of Na ions from the exchange complex by those of Ca^{2+} ions introduced in the system by the dissolution of gypsum applied. Similar results were obtained by Manchanda *et al*. (1985), Choudhary *et al*. (2007) and Tikkoo *et al.* (1997).

Available N, P and K

The data presented in Table 3 revealed that the available N in soil is significantly increased with the application of gypsum and zinc over no gypsum and zinc applied. The interactive effect of gypsum and zinc in this present investigation was non–significant. The available N was found highest in case of G_{100} (132.2 kg ha^{-1}) treatment because of addition of gypsum reduced the soil pH. Further, the neutralization of sodicity improved soil microbial activity leading to more nitrogen mineralisation and available N may be increased due to the fixation of N by the microbes, the result is in agreement with Singh *et al.* (2009). The zinc application also produced significant increase in available N and the highest available N recorded in Zn_{25} (124.5 kg ha^{-1}) compared to control (118.7 kg ha^{-1}). Similar result obtained by Rasouli *et al.* (2013) and Adkine *et al.* (2017). The interactive effect of gypsum and zinc in this present investigation was non–significant. Available P content of soil was enhanced significantly with gypsum application over no gypsum and highest and lowest value for available P was found in the G_{100} (17.6 kg ha^{-1}) and G_0 (9.92 kg ha^{-1}) treated plots and this may be due to the fact that the decrease in soil pH resulting in more availability of native and applied P. The available

Table 3. Effect of Gypsum and Zinc application on available N (kg ha^{-1}) and available P (kg ha^{-1}) in soil after harvest wheat crop under sodic water irrigation

Gypsum Level	Level of Zn ((kg ha^{-1})									
	Available N					Available P				
	Control	05	10	25	Mean	Control	05	10	25	Mean
G_0	110.67	112.33	114.00	116.00	113.25	9.00	9.67	10.67	10.33	9.92
G_{50}	115.00	118.00	119.00	121.00	118.25	11.00	11.33	13.00	12.67	12.00
G_{75}	120.00	122.00	123.00	126.00	122.75	13.33	14.00	15.00	16.33	14.67
G_{100}	129.00	131.00	133.67	135.00	132.17	16.00	17.00	18.00	19.33	17.58
Mean	118.67	120.83	122.42	124.50		12.33	13.00	14.17	14.67	
LSD ($p \le 0.05$)	Gypsum Level (G)= 1.01 Level of Zinc (Zn)= 1.01 G × Zn= NS					Gypsum Level (G)= 1.12 Level of Zinc (Zn)= 1.12 G × Zn= NS				

Table 4. Effect of Gypsum and Zinc application on available K (kg ha^{-1}) and Zn in soil after harvest of wheat crop under sodic water irrigation

Gypsum Level	Level of Zn ((kg ha^{-1})									
	Available K					Available Zn				
	Control	05	10	25	Mean	Control	05	10	25	Mean
G_0	163.67	164.00	166.33	168.00	165.50	0.50	0.68	0.72	0.79	0.67
G_{50}	170.33	172.00	175.00	176.00	173.33	0.68	0.92	0.83	0.98	0.85
G_{75}	177.67	178.33	181.00	182.33	179.83	0.86	1.28	1.40	1.30	1.21
G_{100}	184.00	184.67	187.00	188.33	186.00	1.02	1.58	1.65	1.67	1.48
Mean	173.92	174.75	177.33	178.67		0.77	1.11	1.15	1.18	
LSD ($p \le 0.05$)	Gypsum Level (G)= 1.22 Level of Zinc (Zn)= 1.22 G × Zn= NS					Gypsum Level (G)= 0.16 Level of Zinc (Zn)= 0.16 G × Zn= NS				

K (186.0 kg ha^{-1}) was recorded greater in G_{100}, followed by G_{75} (179.8 kg ha^{-1}) and G_{50} (173.3 kg ha^{-1}) than G_0 (165.5 kg ha^{-1}). Application of zinc also significantly increased the available K, a greater value of available K (178.7 kg ha^{-1}) was recorded in Zn_{25} treatment, followed by Zn_{10} (177.3 kg ha^{-1}) and Zn_5 (174.8 kg ha^{-1}) and control (173.9 kg ha^{-1}). The interactive effect of gypsum and zinc in this present investigation was non–significant.

Available S, Zn and Ca

Application of zinc also significantly increased the available S content. The greater values of available S were (51.6 kg ha^{-1}) in Zn_{25} followed by Zn_{10} (50.3 kg ha^{-1}) and Zn_5 (49.14 kg ha^{-1}) as compared with control (47.59 kg ha^{-1}). The interactive effect of gypsum and zinc in this present investigation was found non-significant. Available S content of soil was significantly enhanced with gypsum application over no gypsum and highest and lowest value for available S was found in the G_{100} (60.8 kg ha^{-1}) and G_0 (39.9 kg ha^{-1}) treated plots, it may be due to decrease in soil pH resulting in more availability of native and applied. Similar result were also reported by Singh *et al.* (2009). The data in Table 4 revealed that the available Zn in soil is significantly increased with the application of gypsum and zinc over no gypsum and zinc applied. The available Zn (1.48 mg kg^{-1}) was greater in G_{100} treatment, followed by G_{75} (1.21 mg kg^{-1}) and G_{50} (0.85 mg kg^{-1}) than G_0 (0.67 mg kg^{-1}). Application of zinc also increases the available Zn, the increment was greater in Zn_{25} (1.18 mg kg^{-1}) ≥ Zn_{10} (1.15 mg kg^{-1}) ≥ Zn_5 (1.11 mg kg^{-1}) as compared with control (0.77 mg kg^{-1}). The interactive effect of gypsum and zinc in this present investigation was found non-significant.

Table 5. Effect of Gypsum and Zinc application on available Ca (kg ha^{-1}) and available S in soil after harvest of wheat crop under sodic water irrigation

Gypsum Level	Level of Zn (kg ha^{-1})									
	Available Ca					Available S				
	Control	05	10	25	Mean	Control	05	10	25	Mean
G_0	46.8	48.0	48.0	48.0	47.6	38.60	39.37	40.30	41.27	39.88
G_{50}	54.8	52.0	57.2	57.2	55.2	43.43	44.37	45.30	47.43	45.13
G_{75}	56.0	58.8	61.2	61.2	59.2	49.43	51.53	54.77	55.63	52.84
G_{100}	62.8	64.0	65.2	66.8	64.8	58.90	61.30	60.77	62.13	60.78
Mean	55.2	55.6	58.0	58.4		47.59	49.14	50.28	51.62	
LSD ($p \leq 0.05$)	Gypsum Level (G)= 0.14 Level of Zinc (Zn)= NS G × Zn= NS					Gypsum Level (G)= 0.94 Level of Zinc (Zn)= 0.94 G × Zn= NS				

The data (Table 5) indicated that the zinc application did not significantly influence the available Ca, maximum mean available Ca (58.4 kg ha^{-1}) was recorded under Zn_{25} treatment, followed by Zn_{10} (58.0 kg ha^{-1}) and Zn_5 (55.6 kg ha^{-1}) and minimum available Ca (55.2 kg ha^{-1}) was observed in control treatment. The interactive effect of gypsum and zinc in this present investigation was non-significant. Available Ca content of soil was significantly enhanced with gypsum application over no gypsum and highest and lowest value for available Ca was found in the G_{100} (64.8 kg ha^{-1}) and G_0 (47.6 kg ha^{-1}) treated plots, it may be due to decrease in soil pH resulting in more availability of native and applied Ca. Similar result reported by Singh *et al.* (2009). The application of zinc does not influence significantly to available Ca content of soil.

Conclusions

The results revealed that the gypsum application neutralize the high RSC of irrigation water and helped in the improvement of physicochemical properties of soil which included soil pH, EC, and ESP. Whereas, zinc application did not influence significantly soil properties. Soil available N, P, K, and S, and Zn were significantly influenced by application of gypsum and zinc, increasing application of both gypsum and zinc levels significantly increased their content in the soil. The application of gypsum and zinc improved fertility status of the soil.

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Isolation, Screening and Evaluation of Biocontrol Potential of Rhizobacteria Isolated from Different Agroecologies

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Abstract

Bacteria is the most abundant class of microflora in the rhizosphere, followed by fungi, protozoa, algae, and actinomycetes. A group of beneficial bacteria thriving near the roots of plants are referred to as plant growth-promoting rhizobacteria (PGPR). Hence, the rhizospheric soil of monsoonal crops (rice, maize, sorghum, pearl millet, and pulses, such as black gram and green gram) and winter crops (wheat, wild oats, mustard and barley) were collected from different experimental fields of Karnal and Panipat for the isolation of rhizospheric bacteria. The dual culture plate method was used to assess the biocontrol potential of purified isolates against the fungal plant pathogens. A total of 184 isolated bacteria were subjected to qualitative screening for their biocontrol activity against seven test phytopathogenic fungi on PDA medium by single streak method. Most of the isolates demonstrated broad-spectrum activity in opposition to the test fungi. Out of the total 184 isolates, sixty-six isolates showed good biocontrol activity against the test phytopathogenic fungi and only 20 isolates exhibited desirable growth inhibition. The rhizobacteria demonstrated secretion of compounds showing biocontrol activity like siderophores, HCN, ammonia, hydrolytic enzymes like cellulase, chitinase etc. Thus, rhizobacteria can be utilized as a significant alternative to conventional farming practices for better and sustainable agricultural output, maintenance of food security, increased yields, effective plant protection, and the agro-economic industry

Key words: Beneficial bacteria, Plant growth-promoting rhizobacteria, Biocontrol potential, Siderophores, Hydrolytic enzymes

Introduction

In the current scenario, extensive growth in the global population has led to the need to increase food production from the already reduced agricultural land. The growing population must be fed in a way that is both healthy and sustainable to guarantee a healthy future for the people. But to fulfil these objectives, farmers are constantly and deliberately using hazardous chemicals to reduce insects, pests and diseases (Wojciechowska *et al*., 2016). Therefore, biological control has been globally approved as a precious approach to pest management for many years. "Biological control" is the use of an organism or combination of two or more organisms to reduce the population density of harmful pathogenic organisms and thus includes the control of animals, weeds and microbial diseases (Prathap and Ranjitha, 2017).

The soil environment in which the plant root is present is known as the rhizosphere. It is a region of high microbial activity that creates a constrained nutrient pool from which essential macro- and micro-nutrients can be extracted (Bhatt and Bhatt, 2021). Bacteria are the most abundant class of microflora in the rhizosphere, followed by fungi, protozoa, algae, and actinomycetes (Kour *et al*., 2019; Poria *et al*., 2021). A group of beneficial bacteria thriving near the roots of plants are referred to as plant growth-promoting rhizobacteria (PGPR) (Santoyo *et al*., 2021). The presence of root exudates, which serve as a source of nutrients for microbial growth, makes the microbial population in the rhizosphere noticeably different from that of the environment surrounding it (Zhao *et al*., 2021). Studies have also claimed that the utilization of rhizospheric bacteria as biofertilizers and biopesticides could be a promising eco-friendly approach for sustainable agriculture (Prathap and Ranjitha, 2017). So, taking into consideration the ability to suppress the growth of pathogens by multiple direct and indirect mechanisms and also, to efficiently colonize the rhizosphere, antagonistic rhizospheric bacteria have been reported as a potent strategy to control soil-borne pathogens (Olanrewaju *et al*., 2017).

The rhizobacterial community has several critical responsibilities like improving soil texture, nutrient acquisition and assimilation, secretion and regulation of metabolites such as antibiotics, hormones, hydrolytic enzymes like chitinases and cellulases along with various antagonistic compounds like siderophores, HCN, and ammonia. Thus, the role of rhizobacteria is not only restricted to plant growth and development but also in controlling the growth of phyto-pathogenic microorganisms making it one of the active ingredients in the development of biofertilizers (Sudha *et al*., 2022; Sukmawati *et al*., 2021). Hence, the present study aims at isolating several rhizobacteria from the soil of different crops that have been collected from different locations and further screening and evaluating them for their biological control potential.

Material and Methods

All the experiments were conducted at ICAR-Central Soil Salinity Research Institute, Karnal. This study aimed at isolation and screening of rhizobacteria showing biocontrol activity. Chemicals of analytical grade (AR) from Hi-media (India), Sigma chemical company (USA), Merck India Ltd., Mumbai, CDH, SDFCL etc. were used for the study.

Collection of rhizospheric soil: The rhizospheric soil of monsoonal crops (rice, maize, sorghum, pearl millet, and pulses, such as black gram and green gram) and winter crops (wheat, wild oats, mustard and barley) were collected from different experimental fields of Karnal and Panipat.

Phytopathogens: Seven fungal phytopathogens viz. *Fusarium sp.* (*FuS*), *Bipolaris sorokiniana* (*BiSo*), *Aspergillus sp.* (*AsS*), *Fusarium oxysporum* (*FuO*), *Penicillium sp.* (*PeS*), *Aspergillus niger* (*AsN*), *Rhizoctonia solani* (*RhS*) were used in the present study.

Isolation of rhizobacteria from soil: Rhizospheric bacteria were isolated from 10 g soil by serial dilution plating method on tryptone soy agar (Himedia®) agar plates as described (Chandra *et al*., 2020) and incubated at 28 ±2°C for 24 hours. After incubation, morphologically different bacteria were picked with sterile inoculating loop and purified on sterile nutrient agar slants for further study.

Screening of isolates for biocontrol activity against fungal phytopathogens: The dual culture plate method was used to assess the biocontrol potential of purified isolates against fungal plant pathogens.

Qualitative screening of biocontrol activity against the selected fungal pathogens was done by the single-streak method in which a loopful of culture was streaked in a line adjacent to the fungal disc placed at the centre of the plate. The plates were kept for incubation (30°C for 4-5 days). The plate with fungal disc without bacteria was taken as control. The diameter of the inhibition zone around bacteria was measured (in mm) and recorded based on their ability to inhibit the growth of test fungal phytopathogens.

Quantitative screening of biocontrol activity was done by agar-disc method on potato dextrose agar (PDA). The PDA plates were inoculated with a fungal disc (2 mm) at the centre and agar wells (3 mm diameter) were punctured with the help of a sterile cork borer around it. The bacterial isolates were grown for two days in nutrient broth on a revolving shaker (120 rpm; 28°C). After incubation, the bacterial cells were removed by centrifugation (6000 rpm; 10 min at 4°C) and the supernatant obtained was taken for bioassay. 0.2 ml culture supernatant was transferred into each well and kept for incubation (28°C; 4 days). The plates were observed for antagonistic activity of bacterial isolates as clear zones of inhibition around the wells and were determined in millimeters (mm). The inhibition percentage (%) was determined by the following equation (Riungu *et al.*, 2008):

$$\text{Zone of inihibition (\%)} = \frac{\text{Radius of fungal disc (Control)} - \text{Radius of fungal disc (test)}}{\text{Radius of fungal disc (Control)}}$$

Production of antagonistic compounds

Siderophores (iron-chelating compounds)

To identify the capability of isolates to produce iron-chelating compounds, Chrome azurol S (CAS) dye-infused blue agar plates were used. For that, 100 ml of CAS-HDTMA solution was prepared by mixing solution I [CAS-121 mg ml^{-1}; 10 ml of Fe (III) solution (13.5 mg $FeCl_3.H_2O$ + 41.6 µl conc. HCl in 50 ml)] and solution II (72.9 mg HDTMA in 40 ml distilled water). CAS-HDTMA solution and nutrient agar medium were sterilized by autoclaving at 121°C for 15 minutes. To 400 ml of sterile nutrient agar, 100 ml of CAS-HDTMA solution was gently mixed under aseptic conditions, poured into sterile plates and left to solidify. The selected isolates were streaked on the solidified CAS agar plates and incubated (30°C; 5 days). A positive result for siderophore secretion by isolates was the appearance of an orange clear zone around the bacterial growth. The diameter of the clear zone was measured and siderophore production index was calculated by the following equation (Sadiq *et al.,* 2014):

$$\text{Siderphore production index (SI)} = \frac{\text{(Diameter of colony + hydrolytic zone diameter)}}{\text{Diameter of colony}}$$

Hydrogen cyanide (HCN)

The bacterial isolates were cultured on nutrient agar medium amended with glycine (4.4 g L^{-1}). Identical circular-shaped filter papers were immersed in freshly prepared alkaline picrate solution (Picric acid-2.5 g L^{-1}; Sodium carbonate-12.5 g L^{-1}), adjusted into the sterile agar plates and sealed with parafilm. In proportion to the amount of HCN produced, sodium picrate was converted to a reddish-brown substance after incubation (30°C; 48 h). The plates were examined for any kind of change in the filter paper's color. Further, filter paper was dipped in 10 ml of distilled water to elute the color and then, its absorbance was measured at 625 nm (Sadasivam and Manickam, 1992; Reetha *et al.*, 2014).

Ammonia production

The production of ammonia by bacterial isolates was examined by inoculating loopful of culture into sterile peptone water. The inoculated tubes were incubated (7 days; 30°C). The growth suspension was then centrifuged (10,000 rpm; 15 min.) and supernatant was collected. In 2.0 ml of the supernatant, Nessler's reagent (2.0 ml) was added slowly. Development of brown color indicated positive result. The absorbance was measured at 450 nm using UV-Vis spectrophotometer (Cappuccino and Sherman, 1992). Ammonium sulphate was used to prepare standard curve in calculating the concentration of ammonia produced.

Production of cell wall degrading enzymes

Chitinase production

The chitinase production was confirmed by growing the isolates on chitin agar media (Verma and Garg, 2019). For quantitative estimation, the isolates were cultured in production medium supplemented with Chitin (0.5% w/v) under shaking condition. Erlenmeyer flask (250 ml) containing 50 ml of medium was then inoculated by 1 ml culture (24-h old) and incubated (3 days; 37°C; 120 rpm). After incubation, cultures were centrifuged (10,000 rpm; 10 min. at 4°C) and collected supernatant (crude chitinase enzyme) was used to measure chitinase activity at 540 nm by DNS (3, 5- Dinitrosalicylic Acid) assay (Miller, 1959). The standard curve was prepared by using N- acetyl-D-glucosamine (GlcNAc) for the estimation of enzyme activity at 540 nm.

Cellulase production

The bacterial isolates were screened for cellulase production on Carboxymethyl cellulose (CMC) agar. The sterile agar plates were streaked with isolates and kept for incubation (4 days; 30°C). After incubation, the plates were flooded with congo red solution with gentle shaking for 10 minutes (Kasana *et al.*, 2008) followed by washing with distilled water. Cellulose degradation was visualized as clear zones around the bacterial colonies and the diameter was measured.

For quantitative estimation, the strains were inoculated in basal broth supplemented with CMC and incubated at 30°C for 24 h. The cultured broth was centrifuged (8000 rpm; 15 min.) and the collected supernatant was measured for enzymatic activity at 540 nm by DNS (3, 5-Dinitrosalicylic Acid) method (Lynd *et al*., 2002). Cellulase activity was estimated through the detection of the amount of free-reducing sugars produced by hydrolysis of the CMC. Enzyme activity is defined as the quantity of enzyme essential to hydrolyze CMC to release one μmol glucose min^{-1} (Shanmugapriya *et al*., 2012).

Statistical analysis

Samples were divided into triplicates that aided in producing all investigational data as the mean value. The data analysis was done using one way ANOVA method & the mean values were evaluated by using the Multiple Range Test (DUNCAN's) at 5% significant level ($p<0.05$). Indian Statistical Computing Portal for NARS (http://stat.iasri.res.in/sscnarsportal) *via* General Linear Model (GLM) online in SAS (SAS Institute Inc.) was used to perform the statistical analysis of experimental data.

Results and Discussion

Screening of biocontrol activity of bacterial isolates

Total 184 isolated bacteria were subjected to qualitative screening for their biocontrol activity against seven test phytopathogenic fungi on PDA medium by single streak method (Fig. 1). Most of the isolates demonstrated broad-spectrum activity in opposition to the test fungi. Out of the total 184 isolates, sixty-six isolates showed good biocontrol activity against the test phytopathogenic fungi. But maximum isolates failed to manifest the desirable biocontrol activity against all seven pathogens and only 20 isolates exhibited desirable growth inhibition, which is comparable to the earlier reports (Khamna *et al.,* 2009; Kumar *et al.,* 2012; Singh *et al.,* 2014). Results of the present study demonstrated that the majority of bacterial isolates inhibited *Bipolaris sorokiniana (BiSo*) and *Fusarium oxysporum* (*FuO*) more strongly as compared to other test fungi. All the sixty-six bacterial isolates were able to inhibit *BiSo*, forty-one isolates inhibited *FuO* and *Penicillium* sp. The mycelia of *Rhizoctonia solani* was inhibited by thirty-three bacterial isolates while that of *Aspergilus* sp. by thirty-four isolates. *Aspergilus niger* and *Fusarium* sp. were inhibited by twenty-six isolates. The diameter of pathogenic mycelial growth in the presence of bacterial isolates is given in Table 1. The mycelia of plant pathogenic fungi are inhibited by the antagonistic compounds secreted by a variety of strains of bacteria.

The biocontrol activity of different strains of *Bacillus subtilis* and *B. cereus* promoted holes, contraction and decrease in turgidity of the mycelium of *Neocosmospora keratoplastica* and *Rhizoctonia solani*. Additionally, stimulation of morphological aberrations in the hyphae of *Pythium aphanidermatum* due to the action of *Pseudomonas resinovorans* and *P. aeruginosa* were also observed (Al-Daghari *et al.,* 2020). Fungal hyphae were deformed due to the changes in internal osmotic pressure. PGPRs also caused abnormalities in the permeability of cell membrane of *Alternaria hyphae* (Halo *et al*., 2018).

Quantitative estimation of biocontrol activity

The quantitative screening of the twenty bacterial isolates by the agar disc method (Figure 2) demonstrated the blockage of mycelial growth of all seven test pathogens to a desirable extent. Results demonstrated that among all the isolates,

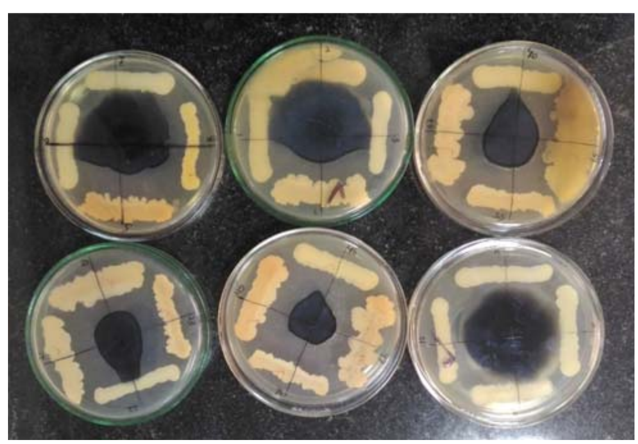

Fig. 1 Screening of isolates by single streak method against *Bipolaris sorokiniana*

Table 1. Colony size (in mm) of pathogenic mycelium in presence of bacterial isolates in contrast with control

S. No.	Isolates	*BiSo*	*FuO*	*PeS*	*RhS*	*AsS*	*AsN*	*FuS*
	Control	3.0±0.06	3.0±0.10	2.0±0.06	3.3±0.06	2.0±0.10	2.5±0.10	2.0±0.06
1.	BP01	2.8±0.06	-	-	3.2±0.12	-	2.4±0.10	-
2.	BP05	2.5±0.10	2.5±0.21	-	-	-	-	-
3.	BP08	2.4±0.15	-	-	-	-	-	-
4.	BP11	1.7±0.15	2.2±0.06	1.4±0.15	2.6±0.15	1.7±0.10	1.9±0.25	1.8±0.15
5.	BP12	2.5±0.23	2.5±0.26	1.9±0.38	-	-	-	1.8±0.12
6.	BP14	2.8±0.21	-	-	-	-	-	-
7.	BP21	2.5±0.21	2.8±0.20	-	2.5±0.15	1.6±0.12	-	-
8.	BP22	2.2±0.25	1.9±0.15	1.6±0.20	2.4±0.12	1.6±0.12	2.3±0.32	1.9±0.21
9.	BP23	2.5±0.21	2.4±0.12	1.8±0.21	-	1.5±0.21	-	-
10.	BP24	2.6±0.12	2.4±0.12	-	-	-	-	-
11.	BP25	2.0±0.10	2.0±0.21	1.6±0.20	2.8±0.15	1.5±0.10	2.2±0.17	1.6±0.06
12.	BP26	2.6±0.10	2.4±0.15	-	-	-	-	-
13.	BP29	2.6±0.29	-	-	-	1.6±0.15	-	-
14.	BP31	2.6±0.10	-	-	-	-	-	-
15.	BP33	2.6±0.29	2.5±0.15	-	2.9±0.21	-	-	-
16.	BP34	2.5±0.20	-	-	-	-	-	-
17.	BP35	1.5±0.21	1.6±0.06	1.1±0.32	2.0±0.26	1.3±0.25	1.3±0.15	1.2±0.17
18.	BP36	2.5±0.23	2.5±0.12	2.8±0.21	-	1.5±0.06	-	-
19.	BP37	2.4±0.10	1.9±0.21	1.9±0.21	-	-	-	-
20.	BP38	2.6±0.15	1.8±0.12	2.6±0.10	-	-	-	-
21.	BP39	2.0±0.06	1.7±0.10	1.0±0.29	2.2±0.21	1.4±0.25	1.3±0.10	1.3±0.10
22.	BP43	1.8±0.21	1.7±0.15	1.7±0.15	2.5±0.26	1.5±0.23	1.5±0.21	1.9±0.21
23.	BP45	2.4±0.15	2.5±0.10	1.8±0.21	-	-	1.5±0.12	1.8±0.21
24.	BP46	1.4±0.10	1.4±0.12	1.3±0.15	2.4±0.06	1.2±0.25	1.6±0.06	1.2±0.12
25.	BP47	2.5±0.15	1.6±0.12	1.5±0.21	-	-	-	-
26.	BP48	2.5±0.06	-	-	2.5±0.23	-	-	-
27.	BP49	2.6±0.10	1.4±0.15	2.4±0.15	-	1.8±0.20	-	-
28.	BP53	2.8±0.20	-	2.0±0.26	-	-	-	1.5±0.10
29.	BP54	2.5±0.20	-	-	-	1.6±0.10	-	-
30.	BP61	2.5±0.15	-	2.6±0.25	2.6±0.25	-	-	-
31.	BP67	1.2±0.21	1.3±0.06	1.2±0.21	2.0±0.15	1.0±0.00	1.3±0.06	1.2±0.12
32.	BP68	2.0±0.15	1.9±0.26	1.3±0.15	2.4±0.21	1.7±0.20	2.0±0.29	1.7±0.06
33.	BP70	2.4±0.21	1.8±0.15	2.0±0.40	-	-	1.8±0.15	-
34.	BP72	1.4±0.12	1.5±0.10	1.0±0.23	2.1±0.21	1.3±0.25	1.8±0.17	1.1±0.06
35.	BP73	2.5±0.10	1.6±0.06	1.9±0.21	-	1.5±0.21	-	-
36.	BP75	2.5±0.15	-	-	-	-	-	-
37.	BP76	2.5±0.15	1.5±0.12	1.5±0.15	-	-	1.7±0.10	-
38.	BP81	2.6±0.26	1.5±0.17	-	1.8±0.17	-	1.5±0.30	1.8±0.21
39.	BP82	2.8±0.35	1.8±0.15	1.5±0.12	-	-	-	1.5±0.15
40.	BP85	2.4±0.12	-	1.6±0.12	-	1.8±0.10	-	-
41.	BP103	2.4±0.20	-	-	-	-	-	-
42.	BP114	2.6±0.06	1.7±0.26	1.2±0.12	2.3±0.12	1.6±0.26	1.6±0.15	1.9±0.26
43.	BP122	2.5±0.21	-	-	-	1.9±0.31	-	-
44.	BP124	1.0±0.06	1.2±0.06	0.8±0.12	2.0±0.26	0.9±0.12	1.2±0.12	0.9±0.10
45.	BP128	2.4±0.10	-	-	-	-	-	-
46.	BP129	2.4±0.17	-	-	2.3±0.32	-	-	-
47.	BP134	2.6±0.21	-	-	2.0±0.29	1.5±0.23	1.8±0.21	-
48.	BP137	1.6±0.17	1.6±0.21	1.3±0.20	2.4±0.12	1.8±0.17	1.5±0.17	1.5±0.12
49.	BP138	1.5±0.15	1.5±0.15	1.2±0.12	2.1±0.21	1.2±0.12	1.2±0.10	1.4±0.12
50.	BP139	1.9±0.15	1.9±0.26	1.5±0.06	2.3±0.15	1.9±0.40	1.7±0.10	1.8±0.23

Contd...

S. No.	Isolates	*BiSo*	*FuO*	*PeS*	*RhS*	*AsS*	*AsN*	*FuS*
51.	BP145	2.5±0.06	-	-	2.0±0.06	-	-	-
52.	BP146	2.3±0.06	1.6±0.15	-	-	1.2±0.12	-	-
53.	BP147	2.4±0.15	-	1.2±0.31	1.5±0.10	-	-	1.5±0.15
54.	BP148	2.4±0.10	-	1.0±0.26	-	-	-	-
55.	BP150	2.5±0.17	-	1.5±0.15	-	-	-	-
56.	BP151	2.6±0.06	1.4±0.15	1.4±0.06	1.5±0.17	1.5±0.17	-	-
57.	BP154	2.5±0.15	-	-	-	-	-	-
58.	BP155	1.9±0.06	2.0±0.10	1.3±0.15	2.5±0.06	1.7±0.15	2.0±0.06	1.6±0.12
59.	BP156	2.4±0.21	1.8±0.15	1.6±0.06	-	1.5±0.12	-	-
60.	BP158	2.5±0.15	1.7±0.15	1.4±0.15	2.3±0.25	1.6±0.06	1.8±0.17	1.9±0.26
61.	BP163	2.6±0.06	-	-	2.2±0.26	1.5±0.23	-	-
62.	BP165	2.5±0.15	-	-	-	-	-	-
63.	BP166	1.9±0.15	1.8±0.20	1.4±0.12	2.7±0.25	1.6±0.00	2.3±0.25	1.8±0.20
64.	BP167	2.4±0.12	-	1.4±0.10	2.0±0.25	-	-	-
65.	BP168	2.5±0.12	2.0±0.25	1.5±0.06	2.5±0.17	1.5±0.12	2.0±0.36	1.6±0.06
66.	BP171	0.9±0.06	1.0±0.06	1.1±0.06	2.0±0.06	0.9±0.06	1.0±0.06	1.3±0.06

Note: *Bipolaris sorokiniana*: *BiSo; Fusarium oxysporum: FuO; Penicillium* sp.: *PeS; Rhizoctonia solani: RhS; Aspergillus* sp.: *AsS; Aspergillus niger*: *AsN*; *Fusarium* sp.:*FuS*; Control: mycelial growth in absence of bacterial isolate.

Fig. 2 Screening of biocontrol activity of isolates by agar disc method *Bipolaris sorokiniana*

BP124 showed significantly ($p<0.05$) highest antagonistic activity against all the phyto-pathogens which was followed by BP171, BP67, BP72, BP35 and BP138. BP124 significantly ($p<0.05$) restrained the growth of *BiSo* up to 72.22% while BP171 and BP67 inhibited the growth up to 71.11% and 65.56%. The growth was inhibited to 52.22% by isolates BP72, BP138 and BP35. In comparison, the growth of *FuO* was significantly ($p<0.05$) inhibited to 65.56% by BP124 followed by BP171 (64.44%), BP67 (61.11%) and BP35 (51.11%). *AsN* was significantly ($p<0.05$) suppressed to 60.00% by BP124 and to 57.33% by BP171 whereas *PeS* was inhibited to 56.67% by BP124 and 50.00% by BP171. The mycelial growth of *RhS* and *FuS* was significantly ($p<0.05$) inhibited to 48.48% and 56.67% by BP124 whereas BP171 inhibited them to 44.44% and 50.00%. *AsS* growth was significantly ($p<0.05$) suppressed to 53.33% by BP124 and to 55.00% by BP171. The inhibition percentage of all the twenty isolates against test phytopathogenic fungi is given in Table 2.

The radial growth is inversely proportional to the percentage inhibition. Biocontrol bacteria undoubtedly exert antibacterial activities through the release of hydrolytic enzymes that break down fungal cell walls as a key defense against infections (Veliz *et al*., 2017). Reports by Sundaramoorthy and Balabaskar (2013) are in similarity with the present results as it showed biocontrol potential of *Pseudomonas fluorescens* against *Fusarium oxysporum*. Similar studies by Zhou *et al* (2021) showed that *Bacillus amyloliquefaciens* suppressed *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Bipolaris sorokiniana* and *Botryosphaeria dothidea*. Yi *et al.* (2021) showed the inhibition activities of a culture filtrate on *B. sorokiniana*, *F. graminearum*, *Rhizoctonia zeae*, *Aspergillus niger* and *A. flavus* which were approximately 53%, 43%, 47%, 36% and 34%, respectively. Lopes *et al.* (2015) reported that

Table 2. Data in terms of inhibition percentage demonstrated by bacterial isolates

Isolates	*BiSo*	*FuO*	*PeS*	*RhS*	*AsS*	*AsN*	*FuS*
BP11	45.56^{D}	22.22^{I}	28.33^{FGH}	23.23^{HI}	15.00^{FGH}	24.00^{HIJ}	8.33^{IJ}
BP22	25.56^{G}	35.56^{GH}	11.67^{J}	29.29^{FG}	20.00^{EFG}	12.00^{L}	6.67^{J}
BP25	32.22^{F}	36.67^{G}	20.00^{HIJ}	15.15^{J}	23.33^{DE}	16.00^{KL}	13.33^{HIJ}
BP35	52.22^{C}	51.11^{BC}	41.67^{BCDE}	39.39^{CD}	38.33^{B}	49.33^{B}	36.67^{CDE}
BP39	33.33^{F}	45.56^{CDE}	36.67^{DEF}	36.36^{D}	33.33^{BC}	44.00^{BC}	35.00^{DE}
BP43	37.78^{E}	43.33^{EF}	15.00^{IJ}	24.24^{H}	30.00^{CD}	36.00^{DE}	6.67^{J}
BP46	45.56^{D}	45.56^{CDE}	36.67^{DEF}	30.30^{F}	38.33^{B}	36.00^{DE}	35.00^{DE}
BP67	65.56^{B}	61.11^{A}	48.33^{ABC}	41.41^{BC}	50.00^{A}	49.33^{B}	45.00^{BC}
BP68	34.44^{EF}	36.67^{G}	35.00^{EFG}	31.31^{EF}	16.67^{EFGH}	20.00^{JK}	16.67^{GHI}
BP72	52.22^{C}	52.22^{B}	45.00^{BCD}	38.38^{CD}	38.33^{B}	49.33^{B}	43.33^{BCD}
BP114	14.44^{H}	43.33^{EF}	36.67^{DEF}	30.30^{F}	18.33^{EFG}	34.67^{EF}	6.67^{J}
BP124	72.22^{A}	65.56^{A}	56.67^{A}	48.48^{A}	53.33^{A}	60.00^{A}	56.67^{A}
BP137	45.56^{D}	44.44^{DE}	40.00^{CDE}	29.29^{FG}	10.00^{HI}	41.33^{CD}	25.00^{FG}
BP138	52.22^{C}	50.00^{BCD}	41.67^{BCDE}	35.35^{DE}	36.67^{BC}	28.00^{GHI}	28.33^{EF}
BP139	34.44^{EF}	34.44^{GH}	21.67^{HI}	31.31^{EF}	3.33^{I}	33.33^{EFG}	10.00^{HIJ}
BP155	34.44^{EF}	30.00^{H}	33.33^{EFG}	25.25^{GH}	13.33^{GH}	20.00^{JK}	13.33^{HIJ}
BP158	17.78^{H}	37.78^{FG}	28.33^{FGH}	30.30^{F}	21.67^{EF}	29.33^{FGH}	5.00^{J}
BP166	35.56^{EF}	37.78^{FG}	23.33^{HI}	19.19^{IJ}	13.33^{GH}	11.33^{L}	8.33^{IJ}
BP168	17.78^{H}	33.33^{GH}	26.67^{GH}	24.24^{H}	23.33^{DE}	22.67^{IJ}	18.33^{GH}
BP171	71.11^{A}	64.44^{A}	50.00^{AB}	44.44^{AB}	55.00^{A}	57.33^{A}	50.00^{AB}
CV (%)	6.38	7.78	17.27	8.53	15.34	11.58	22.08

Note: *Bipolaris sorokiniana: BiSo; Fusarium oxysporum: FuO; Penicillium* sp.: *PeS; Rhizoctonia solani: RhS; Aspergillus* sp.: *AsS; Aspergillus niger: AsN; Fusarium* sp.: *FuS*. Means with different capital letters within same column were found significantly different ($p<0.05$) using DUNCAN's Multiple Range Test.

six unrelated strains of *Saccharomyces cerevisiae* inhibited the pathogenic fungal growth by secreting hydrolytic enzymes after being in contact with the cell wall.

Production of antagonistic compounds

Siderophore production

All twenty bacterial isolates changed the color of the CAS medium from blue to light yellow. This showed that the selected isolates were able to produce siderophores. The siderophore production index (SPI) was calculated (Table 3). Results showed that the significantly ($p<0.05$) highest SPI value was observed for strain BP124 (10.01) followed by BP171 (9.63), BP67 (9.35) and BP11 (8.99) in five days of incubation while significantly ($p<0.05$) lowest SPI value was observed for BP155 (4.13) in the same incubation time.

Rhizobacteria secrete siderophores to deprive phytopathogenic microorganisms of the necessary iron in the rhizosphere micro-environment, hence preventing their hyphal development. This is done to stay alive in the intensely competitive micro-ecological subdivision of the rhizosphere (Berendsen *et al*., 2012). Additionally, the results of Ferreira *et al.* (2019) and Martins *et al.* (2018) are comparable with the present results. One of the key processes for stimulating plant growth & and suppressing disease is the generation of siderophores by biocontrol agents as well as plant growth-promoting microorganisms (Sayyed *et al.*, 2004). As reported by Aznar and Dellagi (2015), induced systemic resistance (ISR) by rhizobacteria promotes plant growth by releasing siderophores.

Hydrogen Cyanide (HCN) production

After incubation, the plates were examined for any kind of change in the filter paper's color. Out of twenty selected bacterial isolates, 19 strains except BP22 produced HCN. The color of the filter paper changed from yellow to brown. The brownish color of HCN produced was eluted. The absorbance was measured at 625 nm. Results showed that bacterial isolates produced HCN in range 0.04-0.05 (Table 3). The maximum production of HCN was shown by BP124 (0.05) followed by BP171 (0.05), BP67 (0.05) and BP72 (0.05) (Fig. 3).

Table 3. Production of antagonistic compounds

Isolates	Siderophore production index	HCN production	Ammonia production (µg mL^{-1})
BP11	8.99^{ABC}	0.04^{EF}	41.00^{AB}
BP22	8.46^{BCD}	0.04^{CDE}	38.18^{BC}
BP25	8.20^{CDE}	0.04^{CDEF}	36.28^{CD}
BP35	7.90^{DEF}	0.04^{CDEF}	35.20^{CD}
BP39	6.85^{GHI}	0.04^{EF}	28.26^{FGH}
BP43	6.21^{HIJ}	0.04^{EF}	24.59^{HIJ}
BP46	5.94^{IJ}	0.04^{F}	23.52^{IJK}
BP67	9.35^{AB}	0.05^{BC}	43.17^{A}
BP68	6.82^{GHI}	0.04^{EF}	29.53^{EFG}
BP72	6.77^{GHI}	0.05^{BCD}	27.12^{GHI}
BP114	6.54^{GHIJ}	0.04^{EF}	25.81^{GHI}
BP124	10.01^{A}	0.05^{A}	44.83^{A}
BP137	5.67^{JK}	0.04^{F}	23.14^{IJK}
BP138	4.81^{KL}	0.04^{F}	21.49^{JK}
BP139	4.35^{L}	0.04^{F}	20.25^{K}
BP155	4.13^{L}	0.04^{F}	19.49^{K}
BP158	6.15^{HIJ}	0.04^{EF}	28.74^{EFG}
BP166	7.42^{EFG}	0.04^{DEF}	32.82^{DE}
BP168	7.07^{FGH}	0.04^{DEF}	32.28^{DEF}
BP171	9.63^{A}	0.05^{B}	43.83^{A}
Mean	7.06	0.04	30.98
CV (%)	8.82	3.55	8.10

Means with different capital letters within same column were found significantly different ($p<0.05$) using DUNCAN's Multiple Range Test.

HCN is a volatile bioactive compound that many rhizobacteria produce due to its strong inhibitory impact on a variety of pathogenic species. Voisard *et al* (1989) and Stutz *et al* (1986) have also demonstrated the function of HCN in the prevention of disease in a variety of crops. According to findings by Alemu (2016), numerous bacterial genera including species of *Bacillus, Pseudomonas, Alcaligenes, Aeromonas* and *Rhizobium* are capable of producing HCN.

Studies by El-Rahman *et al.* (2019) have also demonstrated that it blocks electron transport chain and interferes with the liberation of energy to the cell which causes the death of organisms. The biological control of pathogens is thought to be significantly influenced by the synthesis of HCN by rhizobacteria. Because of its high toxicity to phytopathogenic strains, HCN is recurrently employed as a biocontrol agent in agricultural systems. It is also used to chelate metal ions and is in some way responsible for increasing the accessibility of phosphate (Rijavec and Lapanje, 2016).

According to a variety of surveys by Kamei *et al.* (2014), *Pseudomonas* and *Bacillus* sp. create HCN for promoting plant establishment. Olanrewaju *et al.* (2017) noted that even a small amount of HCN released by the bacterium enhanced the efficiency of antifungal compounds used to treat fungal diseases, preventing the fungus from developing resistance to the specific antifungal consideration. Therefore, it appears that the HCN produced by rhizobacteria works in combination with other biocontrol strategies used by the same bacterium.

Ammonia production

All the bacterial isolates were able to produce ammonia as indicated by the change in color of the medium to brown. All the 20 isolates produced ammonia in the range 19.49-44.83 µg mL^{-1} (Fig. 4). Five bacterial isolates were significantly ($p<0.05$) highest producers of ammonia namely BP124 (44.83 µg mL^{-1}), BP171 (43.83 µg mL^{-1}), BP67 (43.17 µg mL^{-1}), BP11 (41.00 µg mL^{-1}) and BP22 (38.18 µg mL^{-1}). The significantly ($p<0.05$)

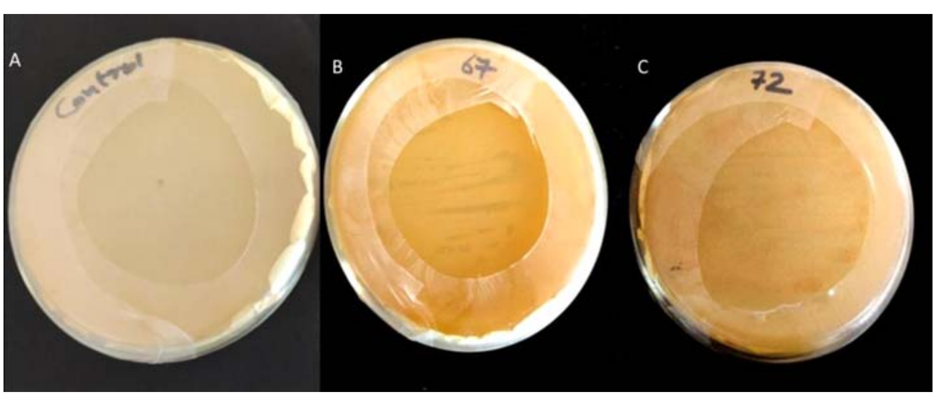

Fig. 3 HCN production by bacterial isolates

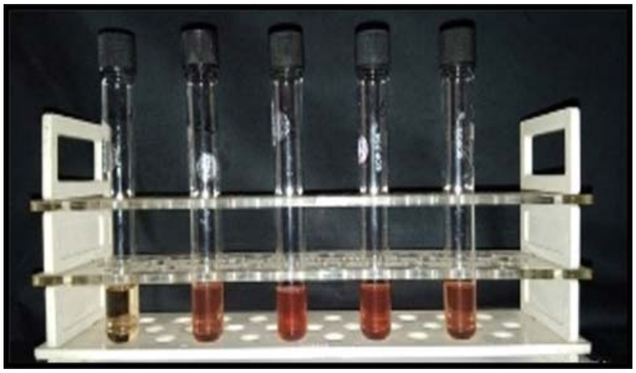

Fig. 4 Ammonia production by bacterial isolates

lowest production of ammonia was observed by BP155 (19.49 μg ml^{-1}) (Table 3).

One of the crucial characteristics that are linked to the improved plant growth and the biocontrol of disease is the synthesis of ammonia by PGPR (Hyder *et al.*, 2020). The nitrogen supplied by the rhizobacteria is transformed into ammonia which aids in the elongation of roots and shoots in addition to enhancement of the biomass production in the host plants (Marques *et al.*, 2010). Additionally, according to Demutskaya and Kalinichenko (2010), ammonia is created during the nitrogen cycle when nitrogenous compounds (peptones) are broken down into ammonia, which is then released into the soil and absorbed by plants as a nutrient. This leads to improvement in the growth & development of plants. Atmospheric nitrogen cannot be used by plants unless it is transformed to ammonia by the nitrogen fixation process, which is done by soil microorganisms, particularly the rhizosphere bacteria. Hence, studies of Passari *et al.* (2015) are in connection with the present results which show ammonia production in the range of 5.2-54 μg mL^{-1} by antagonistic endophytic actinobacteria along with other plant growth-promoting traits.

Production of cell wall degrading enzymes

Chitinase production

All the twenty isolates were found to produce chitinase enzyme as indicated by the zone produced around the colonies on chitin agar (Fig. 5a). Out of twenty, significantly (*p<0.05*) highest chitinase activity was shown by strain BP124 (0.82 U ml^{-1}) followed by BP171 (0.78 U ml^{-1}) and BP67 (0.77 U ml^{-1}), respectively (Fig. 5b).

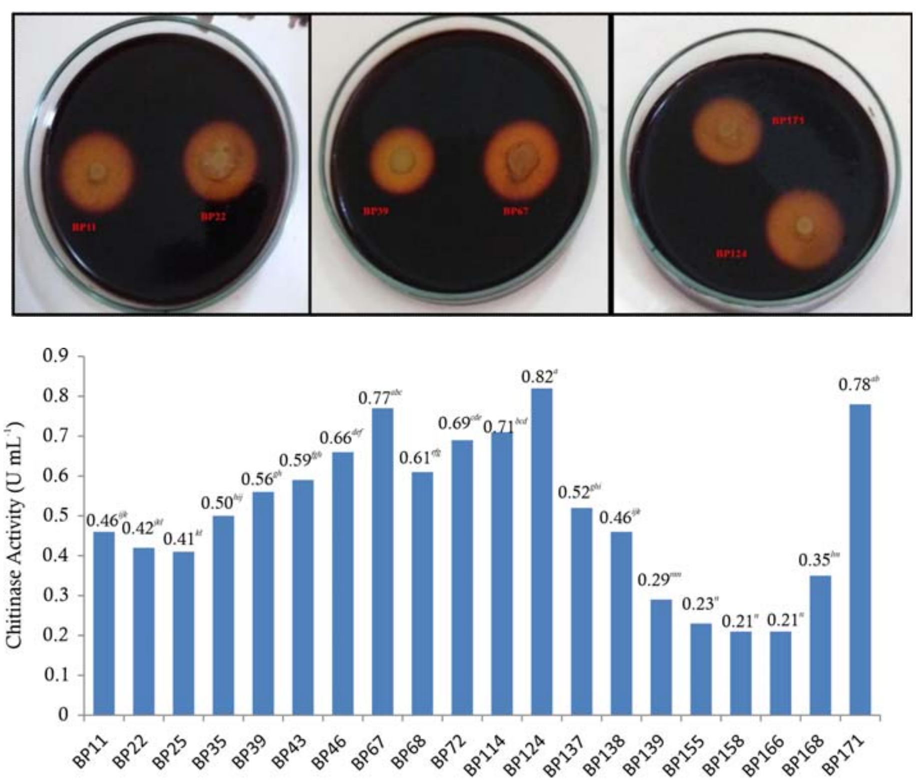

Fig. 5 (a) Clear zones around bacterial growth on chitin agar plates; and (b) Chitinase activity by selected bacterial isolates

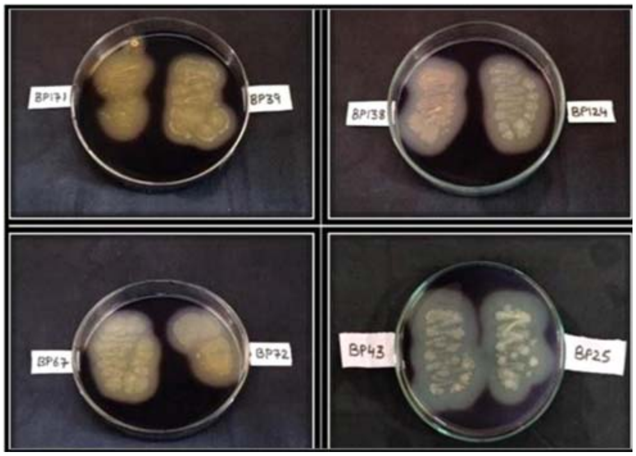

Fig. 6 Clear zones around bacterial growth on CMC agar

Insect and fungal pathogenic chitin can be degraded by complex hydrolytic enzymes called chitinases (Veliz *et al*., 2017). Production of chitinases, cellulases, proteinases, proteinase inhibitors, etc. is triggered by the pathogen's local invasion in many plant species (Kombrink and Somssich, 1995). By utilizing chitin as substrate, clear zones were produced encircling the bacterial colonies due to the hydrolytic action of the chitinase enzyme released by the microorganism (Kuddus and Ahmad, 2013).

Plants also produce chitinase enzymes in response to pathogenic attack. Regarding the current findings, Grover (2012) stated that chitinases function as a crucial toolbox to lower the harmful effects of infection in plants caused by fungal species via undeviating lytic action on their cell walls or by inducing a range of plant defensive mechanisms. Also, in correlation with the results of Verma and Garg (2019), *Bacillus licheniformis* NK-7 chitinase showed biocontrol activity against various phytopathogenic fungi like *Fusarium* sp., *Curvularia* sp., *Aspergillus niger* and *Alternaria* sp.

Cellulase production

By encouraging the breakdown of fungal cell walls, several hydrolytic enzymes such cellulases and chitinases contribute in biocontrol of harmful diseases. The isolated bacterial strains were screened for cellulase enzyme production. The results demonstrated the ability of all the twenty selected isolates to degrade cellulose under aseptic conditions (Fig. 6). Out of twenty, significantly ($p<0.05$) highest cellulase activity was shown by strain BP124 (0.51 U ml^{-1}) followed by BP171 (0.47 U ml^{-1}) and BP67 (0.46 U ml^{-1}), respectively (Fig. 7).

Cellulases are the hydrolytic enzymes produced by different bacteria, symbiotic fungi, protozoa, etc. to breakdown cellulose. Strains of *Bacillus* sp., *Pantoea* sp., *Clostridium thermocellum*, *Pseudomonas fluorescens*, *Ruminococcus albus* and *Streptomyces* spp. are cellulase producers (Atala *et al*., 2014). Secretion of cellulases and chitinases by *Pseudomonas* sp. in the rhizosphere caused

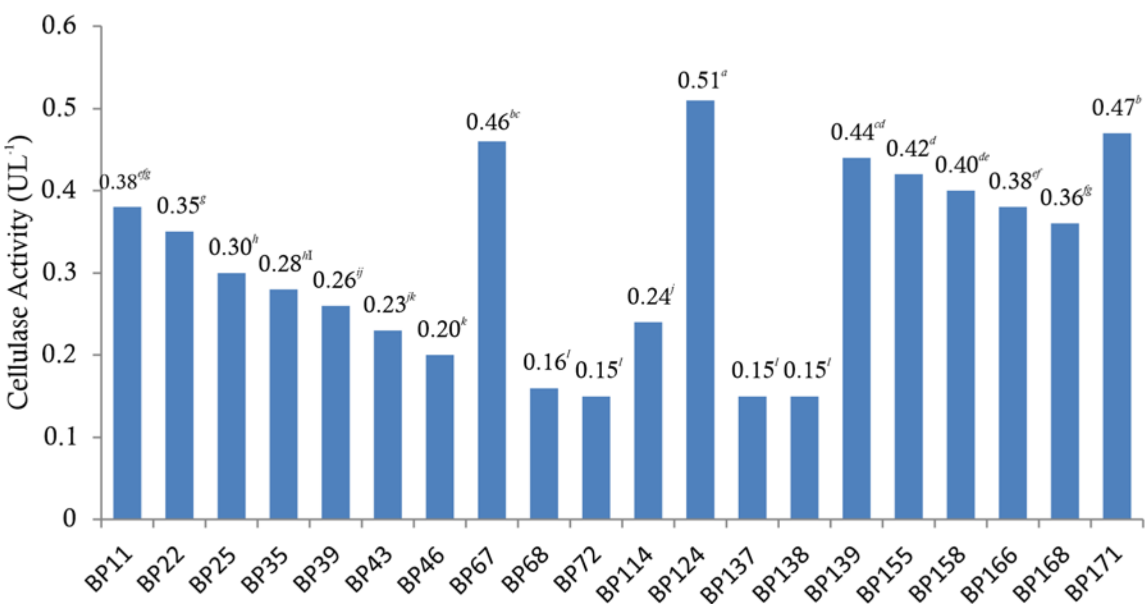

Fig. 7 Cellulase activities by potential bacterial isolates

biocontrol of plant diseases and also, contributed in nodule development by the rhizobia after co-inoculation with soil rhizobacteria (Sindhu and Dadarwal, 2000).

Conclusions

This study highlights the potential of biologically dependent tools, particularly rhizobacteria, to assist in addressing global food production concerns and it is concluded that beneficial interactions between plants and their rhizospheric bacteria involve a complex hormonal signalling network that endogenously controls plant development and defence. The mutual interactions have made it possible for plants and the rhizobacteria they are linked with to exchange several vital compounds showing biocontrol activity like siderophores, HCN, ammonia, hydrolytic enzymes like cellulase, chitinase etc. Effective root colonization is also an essential component of a successful biocontrol rhizobacteria because potential strains can only operate to combat pathogens if they will appropriately colonize the root tissues. Thus, rhizobacteria can be utilized as a significant alternative to conventional farming practices for better and sustainable agricultural output, maintenance of food security, increased yields, effective plant protection, and the agro-economic industry. The commercialization of PGPR as biofertilizers need to get illustrated as these could be the key to sustainable crop productivity and efficient nutrient management.

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Effect of Biosolvent on Salt Contents in Saline Soils and Antioxidant Enzymes of Cotton in the Bukhara region of Uzbekistan

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Abstract

Among abiotic stresses, soil salinization is a very serious environmental stress for plants, reducing yields by up to 20% on irrigated lands worldwide. The study of antioxidant enzymes in the activation mechanisms for salt resistance and the scientific analysis of the salt influence in the biochemical processes of plants leads to the activation of the immune system which serve in quantitative and qualitative increase of plant productivity. Different biochemical processes which are specifically activated under salt stress in the early stages of plant germination. The activities of peroxidase, polyphenol oxidase and phenylalanine ammonia-lease were significantly recorded lower value in roots and leaves of salt-affected crops. The review considers the activity of antioxidant enzymes under salt stress. After washing the saline soil with the biosolvent preparation, the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lease increased in plants exposed to salt stress, which mitigated the negative impact of salt stress. Enzymatic activity was calculated based on Microsoft Excel at the rate of 1 mg g^{-1} in relation to the protein content. The activity of plant oxidoreductase enzymes in saline soil was studied using simple and experimental methods. For simple variants, water was used and the experiment was washed with biosolvent.

Key words: Biosolvent, Antioxidant enzymes, Soil salinity, Cotton, Peroxidase, Polyphenol oxidase, Phenylalanine Ammonia-lease

Introduction

The increase in salinized areas has a detrimental effect on crop yields. This negatively affects the country's economy. Such problems require scientists to create environmentally friendly and effective salt detergents when washing soil salinity, which will be useful not only for the structure of the soil but also for the plant organism. Salinization, which is one of the abiotic stresses, is characterized by an increase for salts in the soil, such as NaCl, $CaCl_2$, $MgCl_2$, Na_2SO_4, Mg_2SO_4, Na_2CO_3 and other carbonates, bicarbonates and nitrites. The presence of these salts in the soil limits the productivity of plants. Salinity is a measure of the number of dissolved salts in the water. It is usually expressed in parts per thousand (ppt), or the number of grams of dissolved salts present in 1,000 grams of water. Cotton agricultural crop is classified as moderately tolerant to salt stress with a salinity limit of 7.7 dS m^{-1} (Munns and Tester, 2008). Salinity sensitivity depends on the stage of plant growth and the type of salt and features of soil and properties in the soil. One of the main problems in the cultivation of agricultural crops is salinity, which is characterized by a direct effect on seed germination, growth, biochemical mechanisms of the plant and, consequently, on its productivity (Munns and Tester, 2008). Three types of salinization are distinguished in the literature: the first is the presence of salts in groundwater, the second is the presence of salts in irrigation water, and the third is transitional salinity (Jabbarov *et al*., 2023). Accumulation of salts in dry areas is faster because of rapid rate of evaporation as compared to humid areas (Stavi *et al*., 2021) and water used for agriculture purpose have more amount of salt (Singh, 2022). It is known, from the literature that salinity directly affects the root shoot of the plant. Saline soils are usually poor in nitrogen and organic matter and rich in salts (Green *et al.*, 2008). Salt concentration depends on the chemical properties, physical properties and soil structure. For example, in 1974 Szabolcs divided saline soils into two types viz., (i) saline soils, the soils that contain more quantity of salts affect the growth of plant and sodium chloride and sodium sulfate are found predominantly in this type of soil and soil electric conductivity is < 8. 2 (cited by James *et al.*, 2011); (ii) sodic soils containing high amount of sodium salts and other name of this soil are known as alkali soil. Initially salt stress inhibits growth and development in the form of osmotic stress, and then this stress continues with ion toxicity (Rahnama *et al.*, 2010). Under the osmotic effect of increased salt accumulation in soils and plants, salinity decreases, and the loss of water by leaves accelerates; therefore, salt stress is also called hyperosmotic stress (Liu and Han, 2021). The osmotic process in the early stages of salt stress causes salt swelling of the root system, which causes various physiological changes such as membrane rupture, imbalance of nutrient uptake through the root, and reactive oxygen species (ROS) detoxification capacity. (Farooq *et al.*, 2015) Most importantly, differences in the activity of antioxidant enzymes, as well as a decrease in photosynthetic activity are because of reduced leaf opening (Munns and Tester, 2008). It is clear that, depending on the level and duration of salt stress, various changes occur in the physiological and morphological processes of the plant, leading to the inhibition of productivity (Gupta and Huang, 2014). Researches have shown that groundwater and irrigation water contain many types of salts, such as calcium chloride, sodium chloride and carbonates, but sodium chloride has a more detrimental effect on cotton yields than other salts (Rengasamy, 2006). It is important to note that salt stress is also considered hyperactive ionic stress. One of the most detrimental effects of salinity is the accumulation of Na^+ and Cl^- ions in plant tissues in soils with high NaCl content. The entry of Na^+ and Cl^- into cells causes a pronounced imbalance of ions, and an increase in the level of Na^+ and Cl^- ions (through tissues) can lead to physiological disorders. Soil salinization is one of the most urgent problems in the modern world. In the world, 25% of arable land is saline to one degree or another, 60% of the lands of Central Asia, and in Uzbekistan, this figure is 60-65%. Existing systems of agro-technological land reclamation measures (traditional salt leaching) require a significant amount of water to wash away water-soluble salts in the surface layers of the soil. In soils with high salinity, these methods do not give the expected effect. Accordingly, in order to improve the agro-reclamation state of irrigated arable lands in agriculture it is important to improve chemical methods of reclamation and create effective preparations. Today, many studies are being carried out in the world on the synthesis of ion-exchange polymers with ion-exchange properties, the study of their chemical structure, molecular size and biological activity, and the creation of biodegradable compositions based on them to improve the agro-reclamation state of irrigated lands and agricultural land. Polycarboxylic acids containing carboxyl groups occupy a special place in terms of high ion exchange properties between ionic polymers. In this regard, the synthesis of water-soluble polycarboxylic acids with a cis-structure and the creation of compositions based on them with effective leaching properties of soil salts of various salinity, as well as chemical soil treatment, i.e., chemical amelioration.

Antioxidant regulation of salinity tolerance

Abiotic and biotic stress in plants can cause overflow, dysregulation, or even disruption of electronic transport chains (ETCs) in chloroplasts and mitochondria. Under these conditions, molecular oxygen ($1O_2$) acts as an electron acceptor, causing the accumulation of ROS. Singlet oxygen (1O), hydroxyl radical (OH), superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2) are highly oxidizing compounds and therefore potentially detrimental to cell integrity (Sachdev *et al.*, 2021). Antioxidant metabolism, including antioxidant enzymes and non-enzymatic compounds, plays an important role in the detoxification of ROS caused by salt stress. Plants grow under the influence of environmental stresses such as salinity, waterlogging and drought, temperature, radiation, mineral deficiency, which qualitatively and quantitatively affect plant productivity. Although assessment of the effects of all various stresses on plants is significant, in view of the running literature, the researchers concentrate their interests on salinity than on other stresses. Salt tolerance positively correlates with the activity of antioxidant enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPC), ascorbate, APX) and glutathione reductase (GR), as well as with the accumulation of non-enzymatic antioxidant compounds (Agarwal and Pandey, 2004). Antioxidant enzyme responses to NaCl stress in cotton. Plant peroxidases are widely distributed in all higher plants, and these enzymes are involved in various plant physiological processes: cross-linking of cell wall proteins and hydroxyproline-rich polysaccharides, both oxidation and polymerization of soluble phenolic compounds, H_2O_2 formation, and chlorophyll degradation and aging. One of the main functions is connected with the role as a part of defense enzyme complex in cells, ensuring detoxification of activated O_2 forms. This function is very important in the formation of metabolic response of plants to different stress factors (Kamran *et al*., 2019). Peroxidase (POX) plays a special role in cell wall strengthening and lignification processes, which are highly resistant to bigot age (Johjima *et al.*, 1999).

Many scientists have studied that polyphenol oxidase plays an important role in lignification reactions as peroxidase (Chen *et al*., 2021) and produces quinones using oxygen, which increases plant resistance to insects and salt stress (Duffey and Stout, 1996). Polyphenol oxidases (PPO) are a group of Cu-containing enzymes that catalyze the oxidation of several phenols to o-quinones. In turn, o-quinones are highly reactive molecules that can undergo non-enzymatic secondary reactions to form brown complex polymers known as melanin has and cross-linked polymers with protein functional groups (Rolff *et al.*, 2011). An increase in the amount of POX and PPO under the influence of salt stress and their participation increases the resistance of the plant to the action of abiotic and biotic stress.

Phenylalanine ammonia-lease is activated by the phenylpropanoid pathway and is a key enzyme in increasing plant stress resistance and its prolongation. In many literatures, it can be observed that the FAL enzyme is activated under the influence of stress, and phenolic compounds or secondary metabolites such as flavonoids are activated in the plant. This is because the involvement of the FAL enzyme in the duration of plant resistance to stressors has been repeatedly studied. FAL as phenolic compounds have strong antioxidant properties, a role in the protection against ROS during salt-water stress adaptation has been long suggested (Akula and Ravishankar, 2011). Previously it was reported that the activity of antioxidant enzymes in plants subjected to salt stress increased (Caverzan *et al.*, 2016).

In order to improve the performance of crops growing under salt stress, it is important to understand how plants cope under such conditions. Salt tolerance of plants is a complex phenomenon that involves physiological, biochemical, and molecular processes as well as morphological. Furthermore, salinity tolerance is unlikely to be determined by a single gene or gene product (Cai *et al.*, 2021). Keeping above facts in mind the present studies were conceptualized.

Materials and Methods

Saline soils (irrigated meadow soil) of the Bukhara district of the Bukhara region of Uzbekistan were selected for this study. Biosolvent increases the efficiency of leaching saline soils (Hudoynazorv, 2018). Cabinet of Ministers of the Republic of Uzbekistan with the requirements of the technological regulations PR-2008 intended for use of biosolvent in all types of irrigation systems as a plant protection and chemicals and have shown an improvement in soil composition. Sample variants were analysed by washing with water. In these two types of soil studies, physicochemical and biochemical analysis of soil composition was carried out using standard methods (Fan *et al.*, 2022; Kaur *et al.*, 2022). At the same time, in order to study the effect of soil salts on the immune system of cotton variety Bukhara-8 which was grown in experimental soils and the activity of antioxidants such as peroxidase (POX), polyphenol oxidase (PPO) and phenylalanine ammonia lysis (PAL) in control variants was determined. Correlations between sample and experimental variants of the roots, stems and leaves of seedlings are compared and analysed.

Analysis of the influence of biosolvent on the process of leaching of salts from the soil in the field was done**.** In field studies, the effectiveness of the use of the preparation "Biosolvent" in saline conditions was analysed in terms of parameters of physicochemical and colloidal properties. In experiments to evaluate the effectiveness of the technology for using a 0.5-10% solution of the Biosolvent preparation, 3 l/he and 5 l/he was tested under conditions of high salinity. In experiments in the Bukhara district of the Bukhara region, the preparation Biosolvent was used by washing the soil.

Methods for studying the physicochemical properties of soils under saline conditions

The magnitude of the electrical conductivity (dS/m) of the soil-water suspension was determined in the ratio EC 1:1 - 1:1 as an objective indicator of the degree of soil salinity - an instrument in an electro conductometer (Russia). The soil pH was determined using a standard pH meter (Germany). Determination of dry residue, HCO^{3-} Cl-, $SO_4{}^{2-}$, Ca^{2+}, Mg^{2+}, K^+ and Na^+ ions in soil samples was carried out using standard indicators and a set of chemical reagents, a photometer, and an electro conductometer. The studied soil sample was weighed on a laboratory scale, mechanically crushed in a mortar and passed through a sieve with a mesh size of 0.25–1 mm.

Determination of carbonates in soil: For the determination of carbonates ($CaCO_3$; $MgCO_3$ $Na_2CO_3 \times 10H_2O$) an approximate method was initially used. In this method, HCl solution (10%) was dropped into a soil sample (irrigation regime of cotton using sprinkler and drip irrigation in the lower Volga region, 2019). In the experiments, salt leaching was determined by the concentration and mechanical composition of salts in the soil at the end of the growing season under normal field conditions. In this case, the removal of salts was calculated according to the following formula, developed by V. R. Volobuev for 1 m of the soil layer, taking into account the water-physical properties of saline soils and the number of salts (Khojiyev *et al.*, 2020).

Here is the coefficient of free salt transfer, $S_{i,}$ S_{adm} are the number of salts in the soil before and after salt leaching (in percentage of the dry mass of the soil).

$N = 10000 \times lg[Si/Sadm]^a \ m^3/ha$

Plant material collection and preparation of extract

As a source of peroxidase, cotton of C-6524 variety were collected from local agricultural field of district Bukhara and carried at 4°C to the laboratory and stored at -20°C until used. In fresh roots, stems and leaves of 12-day-old seedlings of cotton, the activity of peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase enzymes was determined. Cotton was sown on a field treated with Biosolvent at 3 l/he and 5 l/he, for the experiment, and not in a control variant. Each object of the sample was analysed based on the results of three surveys.

Extraction of total soluble protein

One gram of cotton root, stem, leaf tissue per sample was powdered using liquid nitrogen, homogenized in 3 ml extraction buffer (20 mM HEPES, 10% glycerol, 1mM EDTA 100µM PMSF, 5 mM DTT, and 1 mM benzamidine) and centrifuged (16,000 × g, 10 min, 4 °C). Supernatant was collected in fresh tube, dialyzed against 20 mM Tris-Cl and stored at 4 °C for further experiments. The amount of protein was determined by the Lori method. The enzymatic activity was calculated using the Microsoft Excel program at the rate of 1 mg/g in relation to the protein content.

Peroxidase assay

Assay of peroxidase was carried out according to the method of Malik and Singh with certain modifications. In the 2 ml of phosphate buffer (pH 6.0/ pH 7.0), 100 µl of plant extract and 1ml of o-dianisidine solution were added. The reaction was initiated by adding 100 µl of 0.2 × 10> ³M H_2O_2 and the absorbance was read at (460 nm) every 30-second interval up to 5 minutes. The peroxidase activity was calculated using extinction co-efficient of o-dianisidine and the enzyme activity was expressed as unit per mg of protein.

Polyphenol oxidase assay

Polyphenol oxidase activity was determined by the method described previously (Galeazzi and Sgarbieri, 1981). Activity was determined using pyrogallol substrates in absorbance at 494 nm. For enzyme activity, 1 mL of the extract was placed in a cuvette containing pyrogallol substrate and 50 µL enzyme extract was added. The blank sample contained the same concentration of solution, except for 50 µL enzyme extract in 1 ml. One unit of PPO activity was defined as the amount of enzyme causing an increase in absorbance of 0.001 per minute in 1 mL reaction mixture.

Phenylalanine ammonia-lease assay

Assay of PAL was performed using the method (Gómez Vásquez et al., 2004) by using the optimal pH (8 for PAL) of these enzymes. The reaction mixture consisting of 0.5 ml of enzyme extract and 150 mM of L-phenylalanine or L-tyrosine was adjusted to 3 ml with the extraction buffer. Incubation was done at 40 °C for PAL for 30 min. PAL activity was determined at 290 nm, following the formation of E-cinnamic acid. Specific activity of enzymes was expressed acid formed per minute per milligram of protein (mmol. min^{-1} mg^{-1} of protein).

Results and Discussion

Looking at the process of washing with Cl ions, there was a significant difference in the experimental variants compared to the control, but very significant changes were observed in the experimental variant in soil samples taken at a depth of 0-30 cm (Table 1).

Table 1. Dynamics of changes in the number of ions in the soil under the influence of the biosolvent

Ions	Experiment parameters (Indicators)	Control (Average of 3 points)			Experiment 3l/ha (Average of 3 points)			Experiment 5l/ha (Average of 3 points)		
		0-30	30-70	70-100	0-30	30-70	70-100	0-30	30-70	70-100
		Horizons (cm)								
HCO_3^-	Before washing mg/eq	0.010	0.009	0.009	0.010	0.009	0.010	0.000	0.000	-0.019
	After washing mg/eq	0.012	0.011	0.011	0.009	0.010	0.010	-0.002	-0.001	-0.021
	Change (%)	13.7	20.4	19.1	-11.5	4.3	4.2	-25.3	-16.2	-23.3
Cl^-	Before washing mg/eq	0.031	0.033	0.029	0.040	0.037	0.035	0.009	0.005	-0.064
	After washing mg/eq	0.020	0.025	0.025	0.012	0.014	0.018	-0.008	-0.011	-0.043
	Change (%)	-22.2	-17.7	-5.5	-67.8	-59.6	-42.3	-45.7	-41.9	47.8
SO_4^{2-}	Before washing mg/eq	0.466	0.538	0.474	0.451	0.481	0.438	-0.015	-0.056	-0.912
	After washing mg/eq	0.352	0.382	0.355	0.259	0.301	0.305	-0.093	-0.082	-0.659
	Change (%)	-11.6	-21.8	-16.6	-39.2	-35.2	-26.5	-27.7	-13.4	43.2
Ca^{2+}	Before washing mg/eq	0.086	0.105	0.093	0.130	0.142	0.119	0.044	0.037	-0.212
	After washing mg/eq	0.066	0.064	0.059	0.051	0.054	0.050	-0.015	-0.010	-0.109
	Change (%)	-15.7	-32.5	-24.4	-59.5	-60.5	-54.0	-43.8	-28.0	78.5
Mg^{2+}	Before washing mg/eq	0.052	0.057	0.049	0.022	0.024	0.023	-0.029	-0.034	-0.072
	After washing mg/eq	0.041	0.048	0.042	0.034	0.037	0.036	-0.008	-0.011	-0.078
	Change (%)	-4.4	-2.9	5.7	55.5	74.7	72.8	59.9	77.6	-78.5
Na^+	Before washing mg/eq	0.068	0.071	0.069	0.069	0.071	0.067	0.001	0.000	-0.136
	After washing mg/eq	0.031	0.045	0.049	0.020	0.031	0.041	-0.011	-0.014	-0.091
	Change (%)	-48.5	-34.9	-25.8	-69.6	-56.3	-34.2	-21.1	-21.3	60.0
K^+	Before washing mg/eq	0.018	0.016	0.013	0.021	0.017	0.013	0.003	0.001	-0.027
	After washing mg/eq	0.015	0.014	0.012	0.012	0.010	0.009	-0.003	-0.003	-0.021
	Change (%)	-1.5	-5.5	-0.2	-44.1	-33.9	-21.1	-42.5	-28.4	21.4

The data presented in Table 1 show the positive effect of the Biosolvent on the chemical composition of the soil. Biosolvent studies in saline soils convincingly showed the significant changes in the composition of Cl-, SO_4^{2}- Ca $^{2+}$, Mg $^{2+}$, Na^+, and K^+ in the soil for control and experimental variants. Particularly high differentiation was observed in soils obtained at a depth of 30–70 cm. Therefore, in the experiments it was noted that under the conditions of washing (control) of saline soils with ordinary water, the amount of HCO^- in the soil increased slightly, and when washing with the Biosolvent preparation, it did not change or went up steadily. These results indicate that the alkalinity in the composition of the soil does not increase significantly and is a positive condition. Cl^- ions also increased in the control group from 17.7 to 28% after washing in the soil horizon of 30-70 cm and from 59.6 to 63.2% after washing with a Biosolvent. It has been established that the value of this indicator in the soil horizon of 70-100 cm changes from 5.5 to 9.6% and 42.3 to 43.7%, respectively, in the control and Biosolvent group. After washing the saline soil with ordinary water (control), the SO_4^{2}- content in the soil horizon 30-70 cm changed from 21.8 to 29.3% and after washing with a Biosolvent from 35.2 to 45%. After washing with ordinary water (control), the content of Ca $^{2+}$ in the soil horizon 30-70 cm ranges from 32.5 to 39.8%, and when washed with Biosolvent from -60.5 to 60.7%. Experiments have shown that after washing with ordinary water (control), the content of Mg $^{2+}$ in the soil horizon of 30-70 cm increases from 2.9 to 9.8%, and when washed with the Biosolvent preparation, from 52.8 to 74.7%. In the control group, the content of Na^+ in the soil horizon of 30-70 cm increased from 34.9 to 41.1%, and in the washes with the preparation Biosolvent from 56.3 to 64.3%. In the experiments it was found that in the control group, the content of K $^+$ in the soil horizon of 30-70 cm ranges from 5.5 to 16.1%, and in the flush with the preparation "Biosolvent" from 33.9 to 40.4%. Washing with the Biosolvent significantly increases the rate of leaching of ions including Cl^- ions from 35 to 42%, SO_4^{2}- from 13 to 16%, Ca $^{2+}$ from 21 to 28%, Na $^+$ increase from 21 to 23%, Mg $^{2+}$ from 63 to 68% and K^+ from 24 to 28%, which are harmful to plants in saline soils. Biosolvent with water in the ration of 1:10 at the rate of 3l/ha and 5l/ha showed the effectiveness in washing off the salts in saline soils and it saves 2000 m^3/ha of pure water. The Biosolvent had a positive effect on the productive reduction of harmful cations and anions in saline soil, leads to increase of the activity on the biochemical processes of cotton crops.

Figures 1, 2, 3 depict the activity of enzymes which were shifted upwards in the variants of the experiment. The results showed that there was a rise slightly in the activity of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase when applying 3 liters of biosolvent per hectare. In experiments with a flow rate of 5 liters per hectare, it was found that the amount of all enzymes increased significantly compared to control samples.

Figure 1 presents that three types of enzymes were determined from root, stem and leaf of cotton. All enzymes were studied in a control

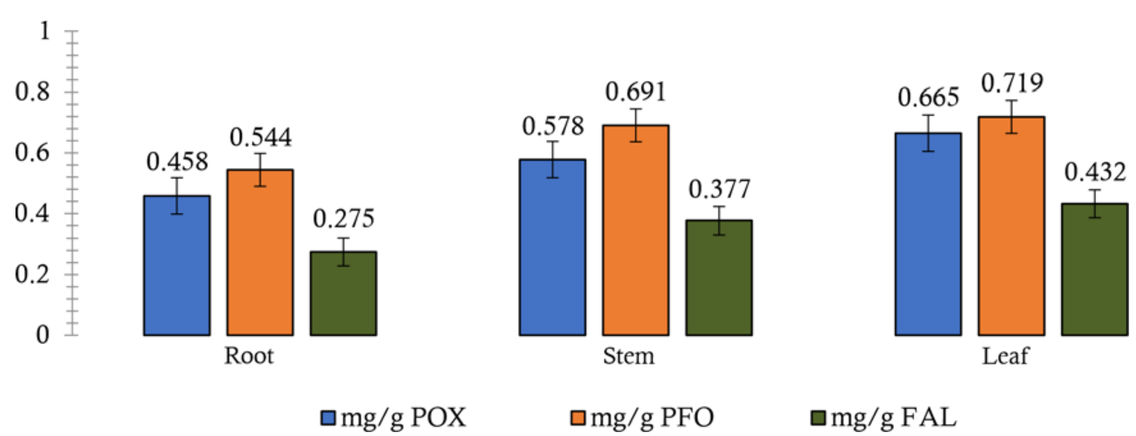

Fig. 1 Activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (control)

variant, for example, control, control 3 l/ha and control 5 l/ha. The focus was to compare the enzyme activities of cotton after leaching with biosolvent. Comparison of the three types of experimental soils can provide a clear scientific conclusion of this study. It is clear from the fig.1 POX activity (blue colour) was 0.458 at root and 0.578 at the stem as well as 0.665 in the leaf. High level of POX was found at the leaf. PFO activity was high at the stem and leaf no major differences was found between two plant parts. In addition, FAl activity were lower from all parts of cotton. However, its activity was slightly rise stem and leaf to compare root system 0.377 and 0.432 respectively.

Figure 2 showed a slight increase in all enzymes compared to the control variant. The soil is washed with biosolvent 3 l/ha and the effect of this preparation on the enzyme activity of cotton variety Bukhara-8 was studied. There was focused on the fact that biosolvent had a positive effect on the soil, reducing the amount of toxic salts. After this, we planted cotton and studied the biochemical process of plants after receiving a reduced titter of salts from the soil. Because if soil properties improve, this can have a positive effect on plant tissue. In this case, biochemical processes were activated first, especially enzyme activity. POX activity was low, but compared to the control variant, there was a difference in the root system. PPO activity increased in the experimental variant by 0.094 and 0.157 at the stem and leaf, respectively. PAL activity was high on the leaf 0.494 and was 0.432 in the control.

The greatest difference was in peroxidase activity can be found from figure 3. At the same time, the enzyme activity in the plant stem increased by 32% compared to the control. It was studied that the activity of this enzyme in the leaf and root of the plant was 32 and 43% higher than in the control, respectively. Another oxido-reductase enzyme, polyphenol oxidase activity, was found to be significantly increased in plant leaf and root by 25%, respectively. However, no significant changes was observed in PFO activity in the plant stem. These results are consistent with the data of other authors on oxidoreductase enzymes in plants under salt stress. Zhang *et al.* (2013) found that *Broussonetia papyrifera* showed changes in the concentration of peroxidase activity under NaCl stress. It is reported that POD activity decreases under conditions of salt stress at a concentration of 100 mM.

Studies of *Glycine max L*. showed that the stress effect of NaCl on stress leads to an increase in the activity of peroxidase and polyphenol oxidase and a decrease in the content of hydrogen peroxide. These results indicate that the number of salts in the soil is not in all cases directly proportional to the activity of oxidoreductase enzymes in plants. These results indicate that the number of salts in the soil is not in all cases directly proportional to the activity of oxidoreductase enzymes in plants. In experiments on different varieties of tomato plants, a non-stationary increase and decrease in the activity of ascorbate peroxidase in leaves under the influence of NaCl stress was revealed in comparison with the activity of the pre-stress

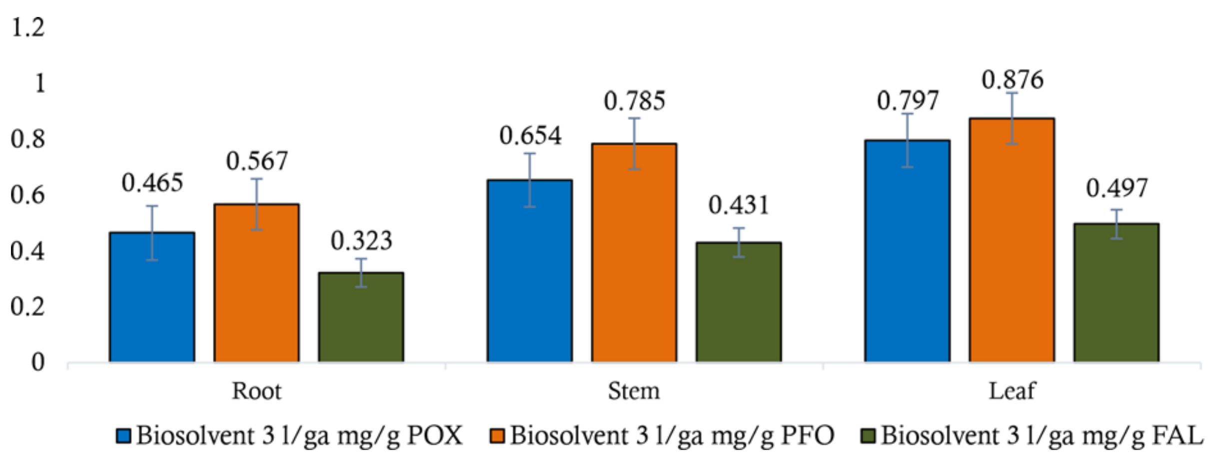

Fig. 2 Activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Experiment 3l/ha Biosolvent)

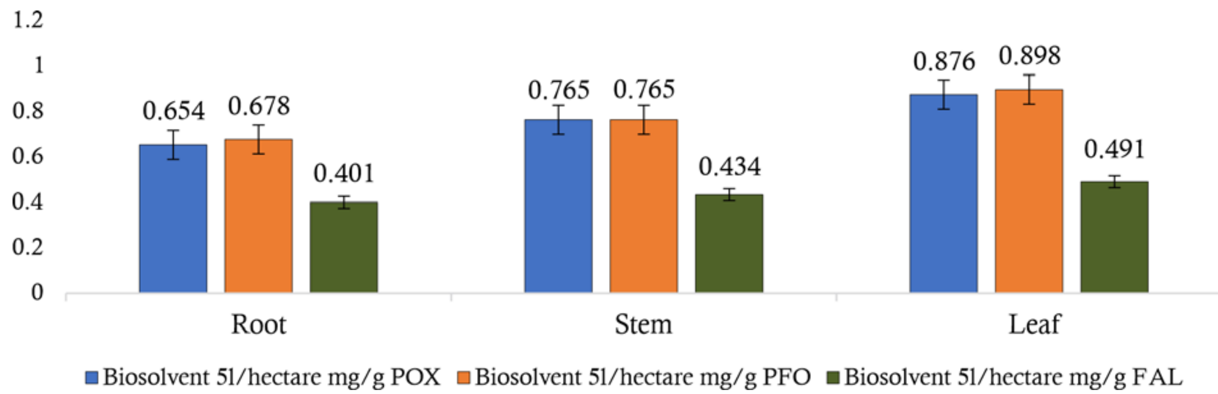

Fig. 3 Activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Experiment 5l/ha biosolvent)

enzyme. It has been established that the activity of enzymes in the root part of the plant periodically decreases in two of the three varieties studied and increases in one. In our study, it was found that the activity of PAL did not change significantly when using a biosolvent at a concentration of 3 l/ha. At a rate of 5 l/ha, no concentration-related changes in stem and leaf results were observed. Only in the root part of the plant, the activity of the enzyme was 46% higher than in the control samples. In contrast to oxidoreductase enzymes, no significant changes in PAL activity were observed in plant leaves and stems. These results are consistent with the following literature data. The expression of the DY523333 PAL gene of the tomato plant was found to be statistically unchanged in plants of salt-tolerant cultivars Ouyang et al. In a study by a decrease in PAL activity was observed under the influence of a single stress in the presence of 150 mM NaCl. However, under the influence of 3-fold stress, a significant increase in PAL activity relative to the control was observed. These results explain that the change in PAL activity in plant tissues under salt stress is concentration dependent. The difference between PAL activity and its m RN AA level is explained by a decrease or inhibition of PAL activity under the influence of high concentrations of trans-cinnamic acid, which is formed under the influence of FAL activity. The reason for the difference between changes in PAL and peroxidase/polyphenol oxidase activity revealed in our study may be related to the above phenomenon.

Soil salinity is a massive abiotic stress, which can decline water potential and induce nutrient imbalances in plants, and these adversely affect plant growth (Mansour and Salama, 2004; Chinnusamy *et al.*, 2005; Genc *et al.*, 2007). The inclusive results of the current study showed that cotton is a stress tolerant crop due to salinity. Full maturation of sensitive variety plants under severe salt stress (in soil containing sodium chloride, and sulfate) made it possible to compare and contrast the responses of tolerant and sensitive varieties. It was tested and selected tolerant and susceptible varieties based on physiological and morphological traits to salt stress in order to explain the underlying physiological, anatomical and antioxidant mechanisms of the defense systems involved. Mechanisms have played a significant and differential role in susceptible and tolerant varieties. Biochemical changes that occur in plants at high concentrations of harmful salts in the soil occur when plants are exposed to salt stress - these include superoxide, hydrogen peroxide and reactive oxygen species . Hydroxyl radicals (Van Breusegem *et al.*, 2001). The activity of peroxidase polyphenol oxidase and phenylalanine ammonia lyase of cotton seeds, formed as a result of salt stress was determined by washing with a bioreactor of 3 l/ha and 5 l/ha. These results are consistent with those of Ben Amor and others. (2006) and Sekmen et al. (2007), where they correlated an increase in PO and PPO activity with tolerance. The activation of the three types of enzymes studied in cotton under the influence of salt stress, especially after washing the soil with biosolvent, showed good results. These data did show that cotton under salt stress provides salt tolerance or adaptive function.

Conclusions

The preparation of "biosolvent" has a high effect on the leaching of salts in the 0-30 cm soil horizon, a good effect on the 30-70 cm horizon and a decrease in relative efficiency on the next 70-100 cm horizon. The results of studying the individual impact of the composition of biosolvent and its components on soil salts and the size of the soil structure allow to draw conclusions about the mechanism of action of the composition. Studies have shown that the introduction of the preparation "biosolvent" in the form of a solution into the saline soil layer and subsequent irrigation significantly reduces the number of salts in the soil rhizosphere of plants. Laboratory studies have shown that under the influence of the "biosolvent" preparation, the content of sulfates in the soil under saline conditions decreased by 23% compared to the control group. It was also established that the salinity of saline soils under the influence of 2-10% of the drug "biosolvent" decreased by 2.4-12 times compared with the control, and the number of salts of general toxic action - by 1.7-12 times. 2.5 times. Magnesium sulfate decreased by 1.9-2.9 times, sodium chloride by 1.1-1.3 times and magnesium chloride by 1.5 times compared with the control.

The biosolvent acts as a conditioner when washing out salts in saline soils, because of which the negative impact of salts on the soil is reduced. In the studies carried out, an inorganic polymer (polymetharylic acid) of a given structure was synthesized and the ratios of its functional groups were determined. In addition, on the basis of an inorganic polymer, a composition has been created that acts at the phase boundary, which ensures the transfer of sparingly soluble salts in the soil into a soluble form, and its physicochemical characteristics have been studied. The effectiveness of the developed polymer composition "biosolvent" for washing soil salts was confirmed based on laboratory and field tests.

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