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In-vitro screening of stress tolerant legume plants

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ABSTRACT

This investigation was aimed to screen stress tolerance of *Vigna radiate, Vigna mungo* and *Vigna unguiculata* by using media containing sucrose and NaCl and modification with Kinetin (KN) and Indole Acetic Acid (IAA). Germination of seeds was observed and biochemical effect was observed by estimation of that amino acids, proline and carbohydrates. It was clear that, the present studies, plant tissue culture technique have enormous potential for the improvement of food crops and that plant tissue culture can equip it with resistance to saline environment.

KEYWORDS

Saline tolerance;
Tissue culture;
Vigna radiate;
Vigna mungo;
Vigna unguiculata.

INTRODUCTION

This is a fact that one third of Indian population is living below the poverty line and 80% of its total population relays on agriculture for their livelihood. Agriculture is the main stay of developing country, most of the developing country suffer set back due to unpredictable monsoon and various stresses with a continuous increase in human population, agriculture is being forced into marginally producing areas. Additionally many developing countries are witnessing a gradual decline in per capita food production. Most of the world food grains surplus is in developing countries the food imports have become more in developing countries.

Plant growth and development are greatly influenced by various environmental factors such as temperature, light, water and nutrient availability[7] variations in these factors that adversely affect plant growth and development are termed as abiotic stresses. These stress conditions dramatically reduce crop productivity. Therefore, there is a pressing need to make plants more tolerant to such conditions[8]. Improvement of stress tolerance by genetic engineering.

Some of the stresses encountered by plants are drought, salinity, high and low temperatures, ultra violet radiation, excess or insufficient light, heavy metal toxicity, air pollution, anoxia, ozone etc[7,11].

Most abiotic stresses such as salt, extreme temperature and drought (osmotic stresses) eventually cause cellular dehydration and therefore, the responses of plants to these stresses overlap to some extent[1]. Under these conditions plants try to maintain water content by accumulating various solutes that are non-toxic and do not interfere with plant processes and are therefore, called compatible solutes[11]. Some of the compatible solutes are fructans, trehalose, polyols, glycinebetaine and proline. Polyamines, although are not strictly compatible solutes, have been shown to play a pivotal

role in plant stress adaptation[7].

Plant programmed cell death: a common way to die and reported that the sugar and abscisic acid response pathways control three developmental processes. Seed development, germination and seedling growth[4]. The effects of ABA and sugar concentration on developmental processes have demonstrated interactions between signaling pathways that can be additive, antagonistic or synergistic, depending on the process, and concentration and chemical form of the sugar signals[4]. Fructans (Polyfructosylsucroses) function as no structural storage carbohydrates in some important crops such as wheat and barley[6]. Fructans are synthesized in the vacuole from sucrose by the action of 2-fructosyl transferases. Fructans also play a role in drought and cold tolerance as they are soluble and can contribute to the osmolarity of the cell[8].

Species growing in sulfate containing soil synthesize choline-O-Sulfate, β-alanine-betaine accumulates in species grown under saline conditions, and praline-derived betaines are accumulated by species growing in dry environments. It is not yet known why different abiotic conditions should favor the accumulation of different osmolytes in members of one family[2].

Stress physiology have shown that there may be a considerable overlap in the responses to different stresses, for example, the antifungal chitinases and glucanases typical of biotic stress responses[9], and also found in response to cold stress where they function as antifreeze proteins[10]. Similarly, heat shock proteins are widely encountered in different stress responses[5]. This has led to the concept of stress cross tolerance where it has been shown that exposure to, oxidative stress may result in tolerance to pathogen stress[3].

MATERIALS AND METHODS

Plant material used

Three different types of legume plants vigna *radiata* (green gram,var.PS16) *vigna mungo* (black gram.var.T.9) and *vigna unguiculata* (cow pea, var. mysore long) were used for the study of salt tolerance.vigna rdiata, vigna mungo and vigna unguiculata were obtained from mahyco seeds company. The seeds of their plants were subjected to different salt concentration to test for their salt tolerance and growth regulator to check if there is increase in growth along with salt. The seeds were first germinated under water soaked conditions and with different salt concentration to check the salt tolerance. Later the seeds were germinated in MS media containing sucrose and salt and sucrose, salt and growth regulators.

Preparation of salt solutions

The three different seeds were subjected to different salt concentration, which were prepared in the following manner, molecular weight of NaCl=54.8gm.

Amount of salt	Volume (ml)	Concentration
58.4 gm	1000	1M or 1000mM
5.8 gm	100	1M or 1000mM
0.365 gm	250	25 mM
0.73 gm	250	50 mM
1.095 gm	250	75mM
1.46 gm	250	100 mM

About 0.365gm of NaCl was weighed and added to 250ml of media at the rate of 25mM concentration. Dissolve the NaCl and dispense about 30ml of medium to each culture bottle. Close the bottle with a cap and label them for the type of medium.

Preparation of kinetin (KN)

From the 1mg/ml of Kinetin stock, different dilution were made in the following order.

Sl. no.	Sucrose	Kinetin (mg/L)	Volume of medium (mL)	Calculation for amount of KN to be added to 100mL media
1	2.00 %	0.01	100	Stock solution of KN = 1mg/mL
2	2.00 %	0.05	100	0.001ml to 100ml of medium = 0.01mg/L
3	2.00 %	0.1	100	0.1ml to 100 ml of medium = 1.0mg/L

Preparation of indole acetic acid(IAA)

From the 1mg/ml of Indole Acetic Acid stock, different dilution were made in the following order.

Sl.no.	Sucrose	IAA (mg/L)	Volume of medium (mL)	Calculation for amount of IAA to be added to 100mL media
1	2.00 %	0.01	100	Stock Solution of IAA = 1mg/ml
2	2.00 %	0.05	100	0.001 ml to 100ml of medium = 0.01mg/L
3	2.00 %	0.1	100	0.1ml to 100 ml of medium = 1.0mg/L

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Establishment of seed culture

The experiments conducted in plant tissue culture require certain parameters such as healthy seeds, sterilized environment like Laminar Air Flow cabinet and hygenic culture room. Around 80-100 normal and healthy looking seeds were selected

Clean the LAF platform with 80%ethanol. All the requirements like culture media $HgCl_2$, DDW, empty conical flasks; culture tubes etc. were transferred to LAF. Closed the door of LAF and put on the UV tube to sterilize them for 45 minutes. In the mean time healthy seeds were sorted out from the provided black gram seeds, to carry out the experiment. The seeds are washed under the running tap water, for about 15-20 minutes in order to remove dust and other contaminating particles adhered to the seeds. Surface disinfected the seeds by treating with 4% Savlon along with 3-4 drops of Tween-20 by vigorous shaking for about 10-15 minutes. The Seeds were washed again under running tap water in order to remove the traces of surfactants. Further sterilization was conducted in the LAF hood.

After 45 minutes of sterilization put off the UV tube, simultaneously put on the air and light and open the door of LAF chamber. The LAF chamber was arranged for efficient working by keeping the things in right places and making sufficient room for work. Again working platform was swabbed and hands were swabbed with 70% ethanol. The seeds were brought to LAF hood for further sterilization. The seeds were transferred to a sterile conical flask and given a short rinse with 70% ethanol for about 60 seconds to remove the organic layer on the surface of the ex plant in order to make them completely free from microorganisms. Decanted the ethanol and collected them in a brown bottle for other use. Immediately the seeds were treated with 0.05 % $HgCl_2$ for 12-15 minutes with vigorous shaking in order to completely sterilize them and make them free from contamination. The $HgCl_2$ is decanted and then the seeds were washed 4-5 times with sterile double distilled water. Now the seeds are completely sterilized and are ready for inoculation. Put on the burner and warm the collar of the culture bottle. The culture bottle was opened keeping the neck close to the flame. About 8-10 sterile seeds were selected from the conical flask, using a sterile forceps inoculated on the media. The forceps were sterilized with the glass bead sterilizer and cooled in ethanol before use. Closed the lid of the culture bottle, showed it over the flame. The bottles were labeled specifying the name, date, and culture. Once the inoculation was done, all the cultures were transferred to a well maintained culture room for incubation.

All the culture bottles are incubated at uniform standard condition of temperature, humidity, and photoperiod. Any large variation of these conditions drastically affects the in vitro regeneration of explants.The culture room is set at a standard temperature of 25 ±2°C and 60-65% relative humidity. The culture were incubated under 16hrs photoperiod provided with white cool, fluorescent tube lights from a distance of 30-35cm receiving about 3000-5000 LUX light intensity.

RESULTS AND DISCUSSION

Effect of Kinetin on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 25mM NaCl

Gram Used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	Kinetin in mg/Lr				
Vigna radiata	2 %	25 mM	0.01 KN	100 %	10	4	0.51
			0.05 KN	100 %	11	4.5	0.68
			0.1 KN	100 %	9	3.5	0.42
Vigna mungo	2 %	25 mM	0.01 KN	100 %	10.5	2.5	1.48
			0.05 KN	66 %	9.5	2	1.31
			0.1 KN	-	-	-	-
Vigna unguiculata	2 %	25 mM	0.01 KN	100 %	11	4	0.52
			0.05 KN	100 %	3	3.5	0.28
			0.1 KN	100 %	11	4.4	0.57

Discussion: In MS media containing different concentrations of growth regulator is found suitable for the germination of the seeds of all the three varieties at the salt concentration of 25mM.*Vigna radiata* showed highest tolerance at 0.05mg/L and *Vigna unguiculata* Showed highest tolerance at the concentration of 0.l mg/L. whereas *Vigna mungo* Showed less tolerance

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Effect of Kinetin on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 50mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	Kinetin in mg/L				
Vigna radiata	2 %	50 mM	0.01 KN	100 %	10	4	0.51
			0.05 KN	100 %	11	4.5	0.68
			0.1 KN	100 %	9	3.5	0.42
Vigna mungo	2 %	50 mM	0.01 KN	100 %	10.5	2.5	1.48
			0.05 KN	66 %	9.5	2	1.31
			0.1 KN	-	-	-	-
Vigna unguiculata	2 %	50 mM	0.01 KN	100 %	11	4	0.52
			0.05 KN	100 %	3	3.5	0.28
			0.1 KN	100 %	11	4.4	0.57

Discussion : In MS media containing different concentrations of growth regulator is found suitable for the germination of the seeds of all the three varieties at the salt concentration of 25mM.*Vigna radiata* showed highest tolerance at 0.05mg/L and *Vigna unguiculata* Showed highest tolerance at the concentration of 0.l mg/L. whereas *Vigna mungo* Showed less tolerance

Effect of Kinetin on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 75mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	Kinetin in mg/L				
Vigna radiata	2 %	75 mM	0.01 KN	100 %	7.5	6.5	0.34
			0.05 KN	66 %	6	4	0.39
			0.1 KN	-- %	--	--	--
Vigna mungo	2 %	75 mM	0.01 KN	100 %	2	4.5	0.15
			0.05 KN	100 %	6	4.5	0.25
			0.1 KN	100 %	7	3	0.35
Vigna unguiculata	2 %	75 mM	0.01 KN	33 %	3.5	2.5	0.54
			0.05 KN	66 %	10	2	0.68
			0.1 KN	100 %	3	1.5	0.52

Discussion : *Vigna mungo* showed highest tolerance at 0.05mg/L of kinetin at 75mM where as *Vigna unguiculata* and *Vigna radiata* showed less tolerance at the same concentration

Effect of Kinetin on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 100mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	Kinetin in mg/L				
Vigna radiata	2 %	100 mM	0.01 KN	100 %	4	4	0.25
			0.05 KN	33 %	1.5	2.5	0.36
			0.1 KN	100 %	2.3	2.5	0.40
Vigna mungo	2 %	100 mM	0.01 KN	100 %	2	2.5	0.34
			0.05 KN	66 %	2.5	2	0.95
			0.1 KN	-- %	--	--	--
Vigna unguiculata	2 %	100 mM	0.01 KN	100 %	7	4.5	0.32
			0.05 KN	66 %	3	2.5	0.25
			0.1 KN	100 %	4	1	0.26

Discussion :*Vigna unguiculata* showed highest tolerance at 0.01mg/L at 100mM where as *Vigna radiata* and *Vigna mungo* showed less tolerance

Effect of IAA on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 25mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	IAA in mg/L				
Vigna radiate	2 %	25 mM	0.05 IAA	100 %	1.85	2	0.29
			1.0 IAA	-	-	-	-
				-	-	-	-
Vigna mungo	2 %	25 mM	0.01 IAA	100 %	6.5	2.25	0.71
			0.05 IAA	-	-	-	-
			0.1 IAA	33 %	8	1	0.55
Vigna unguiculata	2 %	25 mM	0.01 IAA	-	9.65	-	0.37
			0.05 IAA	66.66%		3.00	
			0.1 IAA	-		-	

Discussion :*Vigna unguiculata* showed highest tolerance at 0.05mg/L at 25mM where as *Vigna radiata* and *Vigna mungo* showed less tolerance

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Effect of IAA on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 50mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	IAA in mg/L				
Vigna radiata	2 %	50 mM	0.01 IAA	100 %	9.65	5.15	0.5
			0.05 IAA	100 %	6.75	3	0.45
			0.1 IAA	100%	6.25	2	0.46
Vigna mungo	2 %	50 mM	0.01 IAA	33 %	6.2	3	0.55
			0.05 IAA	100 %	10.5	4	0.86
			0.1 IAA	66 %	4.25	2	0.85
Vigna unguiculata	2 %	50mM	IAA	100 %	4.1	3.55	0.43
			0.05 IAA	100 %	4.25	3	0.44
			0.1 IAA	100 %	8.4	3.25	0.43

Discussion :*Vigna mungo* showed highest tolerance at 0.05mg/L at 50mM where as *Vigna unguiculata* and *Vigna radiata* showed less tolerance

Effect of IAA on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 75mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	IAA in mg/L				
Vigna radiata	2 %	75 mM	0.01 IAA	100 %	6	5.2	0.55
			0.05 IAA	100 %	4	2.5	0.39
			0.1 IAA	100%	5.5	2.5	0.32
Vigna mungo	2 %	75 mM	0.01 IAA	100 %	5	6.25	1.05
			0.05 IAA	66 %	1.50	2.25	0.47
			0.1 IAA	-- %	--	-	--
Vigna unguiculata	2 %	75mM	IAA	66.66 %	3.75	3.65	0.39
			0.05 IAA	100 %	5.9	3.5	0.35
			0.1 IAA	100 %	4.25	2.25	0.30

Discussion : *Vigna radiata* showed highest tolerance at 0.01mg/L at 75mM where as *Vigna unguiculata* and *vigna mungo* showed less tolerance

Effect of IAA on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose and 100mM NaCl

Gram used	Concentration used			Frquency of growth in %	Average shoot length in cm	Average root length in cm	Fresh weight in gm
	Sucrose in %	Nacl in mM	IAA in mg/Lr				
Vigna radiata	2 %	100mM	0.01 IAA	100 %	4.5	3.9	0.36
			0.05 IAA	100 %	2.5	2.5	0.30
			0.1 IAA	100%	2.5	2.25	0.36
Vigna mungo	2 %	100mM	0.01 IAA	100 %	7.5	6.5	1.41
			0.05 IAA	66 %	2.25	2.9	0.83
			0.1 IAA	66 %	2.25	2.75	0.47
Vigna unguiculata	2 %	100mM	IAA	100 %	3.25	4.25	0.34
			0.05 IAA	100 %	4	3.5	0.28
			0.1 IAA	100 %	5	3	0.36

Discussion : *Vigna mungo* showed highest tolerance at 0.01mg/L at 100mM where as *Vigna unguiculata* and *vigna radiata* showed less tolerance

Effect of salt(NaCl) concentration on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose on 9th day

Verities used	No. of days	Sucrose in %	Conc of Nacl in mM	Frquency of germination	Average shoot length in cm	Average root length in cm	Fresh weight in gm
Vigna radiata	9th Day	2	25 mM	100 %	10.05	7.3	0.56
		2	50 mM	100 %	9.6	7	0.42
		2	75 mM	100 %	7.8	5.2	0.39
		2	100 mM	83.33 %	4.3	6	0.36
Vigna mungo	9th Day	2	25 mM	83 %	9	9.05	0.31
		2	50 mM	100 %	10.5	10.75	0.45
		2	75 mM	100 %	7	6.5	0.35
		2	100 mM	100 %	6.45	6	0.31
Vigna unguiculata	9th Day	2	25 mM	66 %	10	7	0.55
		2	50 mM	100 %	13	9	1.35
		2	75 mM	33 %	9	5	0.75
		2	100 mM	83 %	9.5	5	1.21

Discussion :In MS media containing 2 % sucrose was found suitable to all 3 verities of seeds to germinate properly. *Vigna unguiculata* shows highest tolerance in 50mM of salt concentration where as *Vigna radiata* and *vigna mungo* shows less tolerance

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From the above result it is clear that 2 % sucrose containing different concentration of NaCl gives maximum growth for all the 3 varieties of seeds.

Effect of salt(NaCl) concentration on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose on 16th day

Verities used	No. of days	Sucrose in %	Conc of Nacl in mM	Frquency of germination	Average shoot length in cm	Average root length in cm	Fresh weight in gm
Vigna radiata	16th Day	2	25 mM	100 %	13.25	8	0.72
		2	50 mM	100 %	15.25	9.2	0.84
		2	75 mM	100 %	10	8	0.67
		2	100 mM	100 %	14	9.25	0.74
Vigna mungo	16th Day	2	25 mM	100 %	11.9	13.5	0.63
		2	50 mM	100 %	11.35	11.75	1.0
		2	75 mM	100 %	8.4	8	0.42
		2	100 mM	100 %	13.5	12	0.82
Vigna unguiculata	16th Day	2	25 mM	100 %	15.5	5.1	1.14
		2	50 mM	100 %	12	7.5	1.32
		2	75 mM	100 %	14	4.5	0.88
		2	100 mM	100 %	14	4	1.35

Discussion : From the above result it is clear that *Vigna unguiculata* shows highest tolerance in 25mM of salt concentration of NaCl where as *Vigna radiata* and *vigna mungo* shows less tolerance

Effect of salt(NaCl) concentration on germination of *Vigna radiata, Vigna mungo* and *Vigna unguiculata* in MS media containing 2% sucrose on 24th day

Verities used	No. of days	Sucrose in %	Conc of Nacl in mM	Frquency of germination	Average shoot length in cm	Average root length in cm	Fresh weight in gm
Vigna radiata	24th Day	2	25 mM	100 %	25	18.5	1.50
		2	50 mM	100 %	28.5	20	1.79
		2	75 mM	100 %	19.5	13	0.66
		2	100 mM	100 %	14.5	16.5	0.86
Vigna mungo	24th Day	2	25 mM	100 %	8.5	8.7	0.45
		2	50 mM	100 %	10.5	11	0.58
		2	75 mM	100 %	11.5	8.5	0.45
		2	100 mM	100 %	12.5	8.5	0.85
Vigna unguiculata	24th Day	2	25 mM	100 %	15	8.5	1.08
		2	50 mM	100 %	16	4.5	1.06
		2	75 mM	100 %	16.5	10.3	1.19
		2	100 mM	100 %	14	7	1.29

Discussion : From the above result it is clear that *Vigna radiata* shows highest tolerance in 50mM of salt concentration of NaCl where as *Vigna unguiculata* and *vigna mungo* shows less tolerance

Estimation of proline for 9th day, 16th day and 24th day of *vigna radiata*

Vigna radiata (Green gram) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of praline for the 9th, 16th and 24th day. The seeds of the above obtained plantlets were taken and proline estimation is done using the protocol mentioned in methods. The concentrations of proline were extrapolated using a standard TABLE and the values obtained on the 9th, 16th and 24th day were compared to check for gain/loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.129	0.117	0.024
50 mM	0.134	0.026	0.080
75 mM	0.198	0.039	0.090
100 mM	0.104	0.023	0.031

In the 9th day, the proline content of green gram is in increasing state as the salt concentration of NaCl increases. In 16th day as the salt concentration increases there is a decrease in proline content. In 24th day as the salt concentration increases there is increase in 50mM and 75mM of NaCl but there is a decrease in 25mM and l00 mM of NaCl indicating loss of tolerance.From the above result it is clear that there is gain or build up of salt tolerance by green gram as the concentration of salt increases.It has gained more amino acid at 100 mM of NaCl on 9th day.

Estimation of proline for 9th day, 16th day and 24th day of *vigna mungo*

Vigna mungo (Black gram) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of praline for the 9th,

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16th and 24th day. The seeds of the above obtained plantlets were taken and proline estimation is done using the protocol mentioned in methods. The concentrations of proline were extrapolated using a standard TABLE and the values obtained on the 9th, 16th and 24th day were compared to check for gain/loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.198	0.09	0.110
50 mM	0.342	0.07	0.025
75 mM	0.206	0.103	0.045
100 mM	0.090	0.060	0.014

In the 9th day, the proline content of black gram there is gradual decrease at 25mM 75mM and 100mM of NaCl concentration but increases in 50mM concentration.. In 16th day as the salt concentration increases there is a steady increase in 25mM and 75mM but decrease in 50mM and 100mM. In 24th day as the salt concentration increases there is a loss of proline content in 50mM, 75mM and 100mM concentrationFrom the above TABLE it is clear that there is a gain of salt tolerance by a black gram at 25mM of NaCl concentration at 24th day.

Estimation of proline for 9th day, 16th day and 24th day of *vigna unguiculata*

Vigna unguiculata (cow pea) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of praline for the 9th, 16th and 24th day. The seeds of the above obtained plantlets were taken and proline estimation is done using the protocol mentioned in methods. The concentrations of proline were extrapolated using a standard TABLE and the values obtained on the 9th, 16th and 24th day were compared to check for gain /loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.487	0.143	0.110
50 mM	0.502	0.170	0.126
75 mM	0.462	0.141	0.036
100 mM	0.572	0.188	0.008

In the 9th day, the proline content of cow pea is in steady state as the salt concentration of NaCl increases. In 16th day as the salt concentration increases there is a steady increase in amino acid content. In 24th day.as the salt concentration increases there is a loss of proline content.As a result there is a gain of salt tolerance by cow pea at 100 mM of NaCl at 16th day.

Estimation of amino acid for 9th day, 16th day and 24th day of *vigna radiate*

Vigna radiata (Green gram) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of Free Amino Acids on the 9th, 16th and 24th day. The seeds of the above obtained, plantlets were taken and Total Free Amino Acids estimation is done using the protocol mentioned in methods. The concentrations of free amino acids were extrapolated using a standard TABLE and the values obtained on the 9th 16th and 24th day were compared to check for gain /loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.54	0.547	0.48
50 mM	0.35	0.372	0.817
75 mM	0.55	0.563	1.035
100 mM	0.55	0.562	0.905

In the 9th day, the total free amino acids content of green gram is in steady state as the salt concentration of NaCl increases. In 16th day as the salt concentration increases there is a steady decrease in amino acid content. In 24th day as the salt concentration increases there is again of amino acid content.As a result there is a gain of salt tolerance by green gram at 75 mM of NaCl at 24th day

Estimation of amino acid for 9th day, 16th day and 24th day of *vigna mungo*

Vigna mungo (Black gram) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of Free Amino Acids on the 9th, 16th and 24th day. The seeds of the above obtained, plantlets were taken and Total Free Amino Acids estimation is done using the protocol mentioned in methods. The concentrations of free amino acids were extrapolated using a standard TABLE and the values obtained on the 9th 16th and 24th day were compared to check for gain /loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.355	0.51	0.525
50 mM	0.445	0.60	0.62
75 mM	0.58	0.45	0.8
100 mM	0.31	0.575	0.64

In the 9th day, the total free amino acids content of black gram gradually increases as the salt concentration of NaCl increases but decreases in 100mM concentration. In 16th day as the salt concentration increases there is a steady increase in amino acid content. In 24th day as the salt concentration increases there is again of ammo acid content at 75mM of NaCl.From the above TABLE, it is clear there is a building up of salt tolerance by black gram at 75mM of NaCl at 24th day.

Estimation of amino acid for 9th day, 16th day and 24th day of *vigna unguiculata*

Vigna unguiculata (cow pea) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of carbohydrates on the 9th, 16th and 24th day. The seeds of the above obtained plantlets were taken and carbohydrates estimation is done using the protocol mentioned in methods. The concentrations of carbohydrates were extrapolated using a standard TABLE and the values obtained on the 9th, 16th and 24th day were compared to check for gain /loss of tolerance.

Conc. Of salts	9th Day	16th Day	24th Day
25 mM	0.515	1.295	0.68
50 mM	0.675	1.21	0.495
75 mM	0.77	0.87	0.645
100 mM	0.57	0.945	1.05

In the 9n day, the total free amino acids content of green gram increases as the salt concentration of NaCl increases and there is little decrease at 100mM NaCl. In 16th day as the salt concentration increases there is gradual decrease in amino acid content but it gains more tolerance at 2mM NaCl. In 24h day as the salt concentration increases there is a gain of amino acid content.From the above TABLE, it is clear that there is a gain of salt tolerance by cow pea at 25 mM of NaCl at 16th day.

Estimation of carbohydrates for 9th day, 16th day and 24th day of *vigna radiata*

Vigna radiata (Green gram) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of carbohydrates on the 9th 16th and 24th day. The seeds of the above obtained plantlets were taken and carbohydrates estimation is done using the protocol mentioned in methods. The concentrations of carbohydrates were extrapolated using a standard TABLE and the values obtained on the 9th,16th and 24 day were compared to check for gain /loss of tolerance.

Conc. Of salts	9th Day	16th Day	24th Day
25 mM	0.18	0.335	0.250
50 mM	0.072	0.22	0.227
75 mM	0.135	0.235	0.168
100 mM	0.115	0.155	0.122

In the 9lh day, the carbohydrates content of green gram decreases as the salt concentration of NaCl increases. In 16th day as the salt concentration increases there is decrease in carbohydrates and there is a more gain of carbohydrates content at 25mM of NaCl. In 24lh days the salt concentration increases there is decrease in carbohydrates content.From the above TABLE it is clear that there is building up of salt tolerance at 25mM of NaCl at 16th day.

Estimation of carbohydrates for 9th day, 16th day and 24th day of *vigna mungo*

Vigna mungo (Black gram) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of carbohydrates on the 9th 16th and 24th day. The seeds of the above obtained plantlets were taken and carbohydrates estimation is done using the protocol mentioned in methods. The concentrations of carbohydrates were extrapolated using a standard TABLE and the values obtained on the 9th,16th and 24 day were compared to check for gain /loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.12	0.09	0.13
50 mM	0.21	0.09	0.115
75 mM	0.17	0.138	0.08
100 mM	0.2	0.159	0.07

In the 9th day, there is increase in carbohydrates at 50mM, 100mM of NaCl there is a slight decrease at 25mM and 75mM of NaCl.In 16th day as the salt concentration increases there is a steady increase in carbohydrate content. In 24th day as the salt concentration increases there is a loss of carbohydrate content. From the above TABLE it is clear that there is building up of salt tolerance at 50mM and 100mM of NaCl at 9th day.

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Estimation of carbohydrates for 9th day, 16th day and 24th day of *vigna unguiculata*

Vigna unguiculata (cow pea) seeds were taken and germinated in different NaCl concentrations in 2% Sucrose containing Murashige and Skoog media. The seeds germinated with in 2 days and the plantlets so obtained were used for estimation of carbohydrates on the 9th 16th and 24th day. The seeds of the above obtained plantlets were taken and carbohydrates estimation is done using the protocol mentioned in methods. The concentrations of carbohydrates were extrapolated using a standard TABLE and the values obtained on the 9th, 16th and 24th day were compared to check for gain /loss of tolerance.

Conc. of salts	9th Day	16th Day	24th Day
25 mM	0.185	0.14	0.13
50 mM	0.17	0.225	0.194
75 mM	0.185	0.135	0.15
100 mM	0.4	0.15	0.28

In the 9th day, the carbohydrate content of cow pea is more at 100mM of NaCl and decreases at 25mM, 50mM and 75mM of NaCI. In 16th day as the salt concentration increases there is a steady decrease in carbohydrate content. In 24th day as the salt concentration increases there is a steady decrease of carbohydrate content. From the above TABLE, it is clear that there is building up of salt tolerance at 100mM of NaCl at 9th day.

CONCLUSION

In conclusion we can say that the strategies and protocol we followed in the present study gave a good insight for the improvement of different pulse crops for salinity tolerance and the following conclusion have been drawn.

Vigna-radiata grows well in MS media supplemented with 2% sucrose.In media containing sucrose and salt *Vigna radiata* grows well in 50mM of NaCl at 24th Day.Media containing 2% sucrose, NaCl salt and growth regulator, *Vigna radiate* shows high tolerance at O.05mg/lt of KN and 0.01mg/lt of IAA. Proline content was maximum at 100mM indicating high level of tolerance at 9th day.Free amino acids are found to be maximum at 75mM indicating high level of tolerance at 24th day Carbohydrates are found to be maximum at 25mM indicating high level of tolerance at 16th day.

Vigna mango grows well in MS media supplemented with 2% sucrose.In media containing sucrose and salt *Vigna mungo* grows well in 100mM of NaCl at 16th Day.Media containing 2% sucrose, NaCl salt and growth regulator. *Vigna mungo* shows high tolerance at 0.01mg/L of KN and 0.01mg/L of IAA.Proline content was maximum at 25mM indicating high level of tolerance at 24th day.Free amino acids are found to be maximum at 75mM indicating high level of tolerance at 24th day.Carbohydrates are found to be maximum at 50mM indicating high level of tolerance at 9th day.

Vigna unguiculata grows well in MS media supplemented with 2% sucrose. In media containing sucrose and salt *Vigna unguiculata* grows well in 75mM of NaCl at 24th day.For media containing 2% sucrose, NaCl salt and growth regulator, *Vigna unguiculata* shows high tolerance at 0.01mg/lt and 0.1mg/L of KN and 0.01mg/lt of IAA.Proline content was maximum at 100mM indicating high level of tolerance at 16th day.Free amino acids are found to be maximum at 25mM indicating high level of tolerance at 16th day.Carbohydrates are found to be maximum at 100mM indicating high level of tolerance at 9th day.

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