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In vitro evaluation of mungbean (*Vigna radiata* L.) genotypes under salinity stress conditions

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Abstract
The present investigation was carried out at Department of Botany and Plant Physiology, Faculty of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur (Bihar) during 2016 to evaluate mungbean genotypes against salinity stress under *in vitro* condition. Twenty genotypes of mungbean *viz.* IPM 312-19, MH 560A, Pusa Vishal, Pusa 1602, SML 1781, SML 668, Pusa 1641, HDM 12, TMB 37, TMB 163, Pusa 1632, MH 1142, IPM- 99-125, Pusa 1502, SHIM 14-4, Pusa-1601, OUM 11-5, IPM 409-4, Pant M5 and SMP 16-12 were grown *in vitro* at different salinity levels. On the basis of physiological parameters *viz.*, germination percent, seedling length, root length, shoot length and seedling dry weight contrasting set of genotypes were identified. It is found that with increasing salt stress the germination per cent declined this decline in germination per cent might be due to, salinity creating osmotic stress which prevents the water uptake and by toxic effects of ions on embryo viability as well as oxidative damage. The root length and shoot length also show same trends, this may be due to toxic effect of NaCl and decrease in turgor and cell elongation. The genotypes MH 560 A and IPM 312-19 were identified as relatively tolerant, whereas PUSA 1641and SML 668 were found susceptible genotype.

Keywords: Mungbean, *Vigna radiata* L., genotypes, salinity stress conditions

Introduction

Mungbean (*Vigna radiata* L.) commonly known as green gram, is one of the important legume native to India. It belongs to the genus *Vigna* that is composed of more than 150 species originated mainly in Africa and Asia, the Asian tropical regions having the greatest magnitude of genetic diversity (USDA- ARS, 2012) [19]. The *V. radiata* var. sublobata, the wild progenitor of mungbean, is widely dispersed from West Africa to Northern Australia and Papua New Guinea (Undal *et al*., 2011) [18].

Mungbean often experience abiotic stress and among them salt stress is most common that hampers the growth and yield (Alqarawi *et al*., 2014) [3]. The primary effects of salinity stress are reduction in germination percent, fresh and dry weight of shoot and root, leaf water potential, chlorophyll contents, photosynthesis, respiration and protein synthesis (Ahmad and Prasad, 2012; Rasool *et al*., 2013a; Rasool *et al*., 2013b; Alqarawi *et al*., 2014) [1, 13, 12, 3]. Salinity also cause nutritional disorders in plants which lead to deficiencies of several nutrients and drastically increases Na+ levels (Shahid *et al*., 2013) [15]. Moreover, salt stress causes oxidative stress, through the production of variety of reactive oxygen species (ROS) like, singlet oxygen, superoxide ions, hydroxyl radical, H2O2, etc. These ROS are noxious molecules (Ahmad *et al.*, 2010) [2] and causes deleterious effects on mitochondria and chloroplast by disturbing cellular structures (Naz and Bano, 2013) [10]. Salinity, either of soil or water is a serious problem for agriculture all over the world. Salinity limits the growth and development of plant by altering their morphological, physiological, biochemical attributes and production in most of the arid and semi- arid regions of the world (Mudgal *et al*., 2010; Kandil *et al*., 2012) [8, 7]. Mung bean is thus a sensitive crop to salinity and is adversely affected by salt stress in terms of growth and yield. Considering the adverse effect of salinity stress on mungbean cultivation, it is necessary to search variety of mungbean which has tolerant to high saline condition which can be helpful to the farmers for cultivation of mungbean under saline soil.

Materials and methods

Seed sources and storage conditions

Seed materials for research programme were received from Department of a Plant Breeding and Genetics, Tirhut College of Agriculture, Dholi. Seeds were stored at about 8.0 % seed moisture in a sealed container along with cotton dipped in carbon disulphide as disinfectant.

Seeds were tested for germinability at the time of conduct of experiment and seeds having more than 80.0 % germination were taken for studies.

Preparation of salt solutions
The salt solution was prepared by using NaCl: $CaCl_2$: Na_2SO_4 in the ratio of 7:2:1 (w/v) and electrical conductivity of different salinity levels (4.0 and 8.0 dSm-1) were maintained on Direct Reading.

Screening of genotypes
Approximately uniform and healthy seeds were taken for experimental purpose discarding small, large, insect damaged, immature and shriveled seeds. Thoroughly washed Petri dishes were autoclaved at a pressure of 15 psi (121 °C) for half an hour.

Twenty genotypes viz., IPM 312-19, MH 560A, Pusa Vishal, Pusa 1602, SML Conductivity Meter (Systronic Model- 303). These solutions were used to impose different salinity stress on seed germination SHIM 14-4, Pusa-1601, OUM 11-5, IPM 409-4, Pant M5 and SMP 16-12 were subjected to evaluation at different salinity levels *viz.* 0.0, 4.0 and 8.0 dSm-1 in sterilized Petri dishes lined with blotting papers and kept at 25+2 °C in BOD incubator under controlled conditions. Seeds having radicle length of 2.0 mm was considered as germinated seed. Germination counts were recorded after every 24 hours and the experiment was 1781, SML 668, Pusa 1641, HDM 12, terminated on 8 day. Evaluation was done TMB 37, TMB 163, Pusa 1632, MH 1142, IPM- 99-125, Pusa 1502.

Result and discussion
Twenty genotypes were screened at 0.0, 4.0 and 8.0 dSm-1 salinity levels and based on observations such as on the basis of per cent germination, seedling length, root length, shoot length and seedling dry weight.

germination percent, seedling length, root length, shoot length and seedling dry weight, as recorded in 8-day old seedlings, contrasting set of genotypes were selected.

Germination percent
The data revealed that with increasing salt stress, germination percent declined in all the genotypes (Table1). The mean germination percent in control (0.0 dSm-1) was 93.00 which reduced significantly at each subsequent level of salinity stress till it reached 85.00 at 8.0 dSm-1. Thus, this reduced with increasing salinity stress might be due to, salinity creating osmotic stress which prevents the water uptake and by toxic effects of ions on embryo viability as well as oxidative damage. The similar results have been reported by researchers (Sehrawat *et al*. 2013 and Kandil *et al*. 2012) [14, 7] in mungbean who reported that the increased salinity progressively decreased the germination percent. Low water potential due to solute potential arisen from salinity is a determining factor inhibiting the seed germination (Debez *et al*. 2004) [4]. At the maximum salinity level (8.0 dSm-1), minimum percent reduction was recorded in IPM 312-19 (-4.12) followed by MH 560A (-5.10), and the maximum in SML 668 (-14.29) followed by Pusa 1641 (-14.12).

Seedling length
The of mean data of seedling length revealed that with increasing salt stress length of 8- day old seedling declined in all the genotypes (Table 2). Value of mean seedling length in control (0.0dSm-1) was 21.13 cm which reduced significantly at each subsequent level of salinity stress till it reached 20.67 cm at 8.00 dSm-1. At the maximum salinity level (8.0 dSm-1), minimum percent reduction was observed in MH 560A (17.00) and maximum in SML 668 (37.61).

Table 1: Effect of salinity on percent germination of 8-day old mung bean seedlings

Genotypes	Salinity (dSm-1)			Mean
	0.0	4.0	8.0	
IPM 312-19	97	96(-1.41)	93(-4.12)	95
MH 560A	98	97(-1.40)	93(-5.10)	96
Pusa Vishal	99	92(-7.07)	89(-10.10)	93
Pusa 1602	91	88(-3.30)	84(-7.69)	88
SML 1781	91	87(-4.76)	84(-8.10)	87
SML 668	84	78(-7.14)	72(-14.29)	78
Pusa 1641	85	78(-8.24)	73(-14.12)	79
HDM 12	90	87(-2.34)	81(-9.20)	86
TMB 37	88	85(-3.56)	83(-5.85)	86
TMB 163	93	90(-2.95)	80(-13.62)	88
Pusa 1632	97	93(-3.34)	88(-8.93)	93
MH 1142	96	94(-2.02)	89(-6.52)	93
IPM- 99-125	99	95(-3.51)	89(-9.98)	94
Pusa 1502	87	85(-2.27)	80(-7.70)	84
SHIM 14-4	97	93(-4.14)	89(-8.28)	93
Pusa-1601	90	87(-3.33)	84(-6.73)	87
OUM 11-5	98	95(-3.06)	90(-7.66)	94
IPM 409-4	91	89(-2.20)	85(-6.59)	88
Pant M5	96	92(-4.63)	87(-9.00)	92
SMP 16-12	95	90(-4.55)	87(-7.70)	91
Mean	93	90	85	
Factors	LSD (p=0.05)		SEm	
Genotypes	1.63		0.58	
Treatments	0.63		0.22	
G ×T	2.82		1.00	

Table 2: Effect of salinity on seedling length (cm) of 8-day old mungbean seedlings

Genotypes	Salinity (dSm-1)			Mean
	0.0	**4.0**	**8.0**	
IPM 312-19	30.75	26.52(-13.76)	21.75(-29.27)	26.34
MH 560A	32.17	28.4(-11.72)	26.67(-17.10)	29.08
Pusa Vishal	31.95	28.12(-11.99)	25.56(-20.00)	28.54
Pusa 1602	28.48	24.28(-14.75)	20.28(-28.79)	24.35
SML 1781	29.15	24.92(-14.51)	20.26(-30.50)	24.78
SML 668	26.83	21.65(-19.31)	17.23(-35.78)	21.90
Pusa 1641	26.97	21.78(-19.24.)	17.67(-34.48)	22.14
HDM 12	28.00	23.83(-14.89)	20.43(-27.04)	24.09
TMB 37	27.68	23.47(-15.21)	20.34(-26.52)	23.83
TMB 163	29.92	25.77(-13.87)	21.42(-28.41)	25.70
Pusa 1632	29.67	25.47(-14.16)	20.32(-31.51)	25.15
MH 1142	29.43	25.17(-14.48)	21.25(-27.79)	25.28
IPM- 99-125	30.92	26.70(-13.65)	23.33(-24.55)	26.98
Pusa 1502	27.24	22.95 (-15.75)	18.82(-30.91)	23.00
SHIM 14-4	27.41	23.15 (-15.54)	19.45(-29.04)	23.34
Pusa-1601	27.45	23.23(-15.37)	18.64(-32.09)	23.11
OUM 11-5	28.96	24.73(-14.61)	19.95(-31.11)	24.55
IPM 409-4	27.33	23.08(-15.55)	19.33(-29.27)	23.25
Pant M5	27.83	23.63(-15.09)	20.24(-27.27)	23.90
SMP 16-12	27.57	23.37(-15.23)	20.33(-26.26)	23.76
Mean	21.13	24.51	20.67	
Factors	LSD(p=0.05)		SEm	
Genotypes	0.48		0.17	
Treatments	0.19		0.07	
G × T	0.84		0.30	

Table 3: Effect of salinity on root length of 8-day old mungbean seedlings

Genotypes	Salinity (dSm-1)			Mean
	0.0	**4.0**	**8.0**	
IPM 312-19	9.45	8.68(-8.15)	7.85(-16.93)	8.66
MH 560A	9.64	8.75(-9.23)	7.97(-17.32)	8.79
Pusa Vishal	8.15	7.17(-12.02)	6.53(-19.88)	7.28
Pusa 1602	7.56	6.07(-19.71)	5.71(-24.47)	6.45
SML 1781	7.71	6.39(-17.12)	5.99(-22.31)	6.70
SML 668	6.65	4.97(-25.26)	4.64(-30.23)	5.42
Pusa 1641	6.73	5.08(-24.52)	4.74(-29.57)	5.52
HDM 12	7.42	5.94(-19.95)	5.58(-24.80)	6.31
TMB 37	7.29	5.76(-20.99)	5.35(-26.61)	6.13
TMB 163	7.97	6.71(-15.81)	6.36(-20.20)	7.01
Pusa 1632	7.91	6.56(-17.07)	6.21(-21.49)	6.89
MH 1142	7.81	6.45(-17.41)	6.13(-21.51)	6.80
IPM- 99-125	8.95	7.83(-12.51)	7.19(-19.66)	7.99
Pusa 1502	6.84	5.27(-22.95)	4.94(-27.78)	5.68
SHIM 14-4	6.94	5.49(-20.89)	5.04(-27.38)	5.82
Pusa-1601	7.05	5.55(-21.28)	5.18(-26.52)	5.93
OUM 11-5	7.62	6.29(-17.45)	5.81(-23.75)	6.57
IPM 409-4	6.91	5.31(-23.15)	4.98(-27.93)	5.73
Pant M5	7.32	5.86(-19.95)	5.44(-25.68)	6.21
SMP 16-12	7.15	5.63(-21.26)	5.26(-26.43)	6.01
Mean	7.65	6.29	5.85	
Factors	LSD(p=0.05)		SEm	
Genotypes	0.143		0.051	
Treatments	0.055		0.020	
G×T	0.248		0.088	

Table 4: Effect of salinity on shoot length (cm) of 8-day old mungbean seedlings

Genotypes	Salinity (dSm-1)			Mean
	0.0	**4.0**	**8.0**	
IPM 312-19	22.5	19.44(-13.60)	17.71(-21.29)	19.88
MH 560A	22.53	19.65(-12.78)	18.70(-17.00)	20.29
Pusa Vishal	22.6	19.35(-14.38)	15.22(-32.65)	19.06
Pusa 1602	20.92	18.21(-12.95)	14.57(-30.35)	17.90
SML 1781	21.44	18.53(-13.57)	14.27(-33.44)	18.08
SML 668	20.18	16.68(-17.34)	12.59(-37.61)	16.48

Pusa 1641	20.24	16.7(-17.49)	12.93(-36.12)	16.62
HDM 12	20.58	17.89(-13.07)	14.85(-27.84)	17.77
TMB 37	20.39	17.71(-13.14)	14.99(-26.48)	17.70
TMB 163	21.95	19.06(-13.17)	15.06(-31.39)	18.69
Pusa 1632	21.76	18.91(-13.10)	14.11(-35.16)	18.26
MH 1142	21.62	18.72(-13.41)	15.12(-30.06)	18.49
IPM- 99-125	21.97	18.87(-14.11)	16.14(-26.54)	18.99
Pusa 1502	20.4	17.68(-13.33)	13.88(-31.96)	17.32
SHIM 14-4	20.47	17.66(-13.73)	14.41(-29.60)	17.51
Pusa-1601	20.4	17.68(-13.33)	13.46(-34.02)	17.18
OUM 11-5	21.34	18.44(-13.59)	14.14(-33.74)	17.97
IPM 409-4	20.42	17.77(12.98)	14.35(-29.73)	17.51
Pant M5	20.51	17.77(-13.36)	14.80(-27.84)	17.69
SMP 16-12	20.42	17.74(-13.12)	15.07(-26.20)	17.74
Mean	21.13	18.22	14.81	
Factors	LSD(p=0.05)		SEm	
Genotypes	0.384		0.137	
Treatments	0.149		0.053	
G × T	0.666		0.237	

Table 5: Effect of salinity on seedling dry weight (g plant-1) of 8-day old mungbean seedlings

Genotypes	**Salinity (dSm-1)**			**Mean**
	0.0	**4.0**	**8.0**	
IPM 312-19	0.28	0.23(-17.86)	0.21(-25.00)	0.24
MH 560A	0.13	0.09(-30.23)	0.08(-37.98)	0.10
Pusa Vishal	0.30	0.27(-10.00)	0.25(-16.67)	0.27
Pusa 1602	0.23	0.18(-21.74)	0.17(-26.09)	0.19
SML 1781	0.25	0.21(-16.00)	0.19(-24.00)	0.22
SML 668	0.31	0.27(-12.90)	0.25(-19.35)	0.28
Pusa 1641	0.22	0.17(-22.73)	0.16(-27.27)	0.18
HDM 12	0.20	0.15(-23.47)	0.14(-28.57)	0.16
TMB 37	0.27	0.22(-18.52)	0.20(-25.93)	0.23
TMB 163	0.26	0.21(-19.23)	0.20(-23.08)	0.22
Pusa 1632	0.26	0.22(-15.38)	0.20(-23.08)	0.23
MH 1142	0.29	0.25(-13.79)	0.23(-20.69)	0.26
IPM- 99-125	0.15	0.12(-20.00)	0.11(-26.66)	0.13
Pusa 1502	0.17	0.13(-23.53)	0.12(-29.41)	0.14
SHIM 14-4	0.18	0.14(-22.22)	0.13(-27.78)	0.15
Pusa-1601	0.24	0.19(-20.83)	0.17(-29.17)	0.20
OUM 11-5	0.16	0.12(-25.00)	0.11(-31.25)	0.13
IPM 409-4	0.12	0.09(-26.83)	0.08(-34.96)	0.10
Pant M5	0.21	0.16(-23.81)	0.15(-28.57)	0.17
SMP 16-12	0.19	0.15(-21.05)	0.14(-26.32)	0.16
Mean	0.22	0.18	0.16	
Factors	LSD(p=0.05)		SEm	
Genotypes	0.008		0.003	
Treatments	0.003		0.001	
G × T	NS		0.005	

Conclusions

The overall result of this experiment showed the *Vigna radiata* L. is salt sensitive leguminous crop and severely affected by salt stress that's leads to inhibitory effects of on germination, seedling length, root length, shoot length and seedling dry weight. The genotypes MH 560 A and IPM 312-19 found to be relatively tolerant to salinity stress among all the genotype of mungbean whereas genotypes PUSA 1641and SML 668 found to be most susceptible genotype under different concentration of salinity.

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