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Research Article

Gamma rays induced moisture stress tolerant long root mutant in mungbean (*Vigna radiata* L. Wilczek)

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Abstract:
The mutation breeding programme was undertaken to improve the root characters of variety Samrat in mungbean. It is high yielding and MYMV resistant variety but its shallow root system limits the grain yield under moisture stress. Seeds of variety Samrat were irradiated with 450 Gy gamma rays. One long root mutant (LRM) having root length up to 71cm as compared to average 19.5 cm of Samrat was identified and isolated in M_2 generation. LRM was tested in laboratory using various concentrations of polyethylene glycol and also in the field under simulated water stress condition. With increase in concentration of PEG, water uptake, germination, root and shoot length significantly reduced in both LRM and Samrat. The LRM showed significantly greater water uptake, root and shoot length as compared to control Samrat. In the field experiment, LRM recorded better root length along with yield and yield contributing characters as compared to Samrat. The results indicated that a strong root system of LRM will help the genotype to draw comparatively more water to survive and reproduce normally under drought.

Key words:
Gamma rays, moisture stress, mungbean, PEG-6000, root mutation

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is a major pulse crop grown throughout the year in India. In rainfed areas, the plants are exposed to drought stress at any stage of growth but usually during the later stages, particularly in crop grown in the rice fallows Since the onset of monsoon can not be predicted nor an ample supply of irrigation water provided, the most practical approach is to tackle this situation is by growing cultivars with improved tolerance to drought. The root system plays an important role in the regulation of water uptake and extraction from deep soil layers. A deep and thick root system has been thought advantageous for improved drought. tolerance. Mutation techniques have been widely used in efforts to breed abiotic stress tolerance with some success (Gai *et al*. 2006, Spreeth and De Ronde 2006). So far most of the mutation research in plants were carried out for improvement of the above ground parts. The other half that remains in the soil, that is, the roots has been largely neglected. Only in recent years some works have been started looking for mutations affecting the root characteristics. Deeper root systems are widely associated with plant, abilities to absorb water and nutrients from soil depths. Increasing root hair density and length almost doubled phosphorous uptake (Gahoonia and Nielsen 1997) in barley. Mutations affecting root length, density of root hair, number of seminal roots, root tip and zone of root elongation, gravitropism, have been reported in barley and maize (Szarejko and Maluszynski 1980, Feix et al. 1997). It has been shown that *Phaseolus vulgaris* genotypes having higher root biomass produced higher pod and seed yield at low level of phosphorous (Boutraa et al. 1999).The variety Samrat is high yielding and resistant to Mungbean Yellow Mosaic Virus (MYMV) disease but its shallow root system limits

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the grain yield under moisture stress. Therefore, mutation breeding programme was undertaken to improve the root characters of variety Samrat. Seeds of variety Samrat were irradiated with 450 Gy gamma rays. One long root mutant (LRM) having root length 71cm as compared to average 19.5 cm of Samrat was identified (Fig.1) and isolated in M_2 generation (Dhole and Reddy, 2007). In mungbean, seed germination, early seedling and root growth are considered critical in relation to yield and drought tolerance. It is very important to know the advantage of long root in moisture stress under control and field conditions. Therefore the present study was conducted to investigate the response of long root mutant in terms of water uptake, germination, seedling growth, root length and shoot length under PEG-6000 simulated water stress, to compare the yield and yield contributing characters of long root mutant with the parent variety Samrat under simulated water stress in field conditions.

Material and methods

Seeds of variety Samrat and its long root mutant (M_5 generation) have been taken for the present study. Five external water potentials (-2.5,-5.0, -7.5, -11.0 and -15.0 bars) were prepared by using polyethylene Glycol (PEG)-6000 as per the method of Michel and Kaufmann (1973). Water uptake by seeds was studied by subjecting each lot of 100 seeds to treatments with PEG-6000 solution of different water potentials separately in petri plates with three replications. Seeds were removed at predetermined time interval of 12, 24, and 36 hours, washed with distilled water, blotted dry immediately and weighted. The water uptake by each lot (expressed as percentage of dry weight) was calculated by using the following formula

$$\text{Water uptake} = \frac{W_2 - W_1}{W_1} \times 100$$

Where, W_1- initial weight, W_2 – weights at different treatments

For germination study 100 seeds for each treatment were placed in clean sterilized Petri dishes with blotting paper and moistened with respective PEG-6000 solution and replicated thrice. The seeds were allowed to germinate in an incubator at $28\pm2^{\circ}C$ and germination counts were recorded at 24 hour interval up to 72 hours. Seeds with a radical extension equal to or greater than 2 mm were considered as germinated and counted. Percentage germination was calculated based on the total number of seed germinated per treatment after three days of incubation.

For seedling study, presoaked seeds in respective PEG solutions (control without PEG, -2.5, -5.0, and -7.5 bars) allowed to develop seedling in growth chamber with 80 % humidity and $28\pm2^{\circ}C$ temperature and 12 hours light illumination. The seedlings were grown in thermacol cups (10 x 15 cm). The seedlings were held in the holes made in thermacol sheet by using cotton plugs. The cups were filled with respective concentration of PEG solution. The whole experiment was replicated thrice with 25 seedlings in each treatment. The seedling root length was measured in cm at 3, 5, and 7^{th} day after planting. The number of roots and shoot length were recorded only on 7^{th} day. The data recorded and analyzed by factorial randomized design.In another experiment, the long root mutant and variety Samrat were grown in the field with three replications under simulated water stress condition in Summer-2009 at Gamma Field Facility Section, BARC, Trombay, Mumbai. The irrigation was withheld 30 days after sowing. The observations were recorded on shoot length (cm), number of branches per plant, number of pods per plant, 100 seed weight (g), seed yield per plant (g) and root: shoot ratio. At the time of maturity the field was saturated with water by irrigation to loosen the soil. Each plant was uprooted carefully and root length was measured in cm. The mean values of the above seven traits for the mutant and its parent variety were compared by using Fisher's 't' test (Sharma, 1996).

Result and discussion

The differences for water uptake due to genotype, treatments and interaction (genotype x treatment) were found significant at 12, 24 and 36 hours, except for genotype at 24 hours. The water uptake pattern of both Samrat and long root mutant was found to be almost similar under moisture stress condition (Table1). It was found that water uptake by seeds of both genotypes declined progressively with decreasing water potential. Long root mutant showed better water uptake in respective water potentials in comparison to Samrat. The highest water uptake was observed at 36 hours in both the genotypes under all external water potentials including controls. The rate of water uptake was found fastest from 12 to 24 hours and it was slowed down from 24 to 36 hours. The declining trend of water uptake by seeds under decreasing water potential might be due to reduced diffusivity of seed coat to water at lower water potential. Similar results of progressive decline in water uptake by seeds of different crops with the increasing moisture stress have been reported (Singh and Afria, 1985; Dutta and Bera, 2007). Variation in water uptake between the two genotypes having different potentiality for drought tolerance is in agreement with earlier works (Singh and Singh, 1983; Dutta and Bera, 2007). Osmotic potential and cultivar had a significant effect on germination. The germination percentage was not affected up to -7.5 bars water potential in both genotypes (Fig.2). At -11.0 bars water potential, the germination was reduced up to 70 per cent in Samrat while it was 100 per cent in long root mutant. None of the seed germinated at -15.0 bars in both the genotypes. The reduction in germination under moisture stress might be due to less availability of free water to the seeds during early hours of imbibitions thus leaving the hydrolytic enzyme inactive (Hadas, 1976). Decrease in germination was also reported in mungbean (Dutta and Bera, 2007), soybean (Lata et al., 1998) and chickpea (Singh and Afria, 1985). The differences due to genotype, treatment and interaction (genotype x treatment) were found significant for root length at 3, 5 and 7th day and number of roots and shoot length at 7th day, except genotype for number of roots in PEG induced moisture stress. Root growth in terms of length (Table 2) in response to moisture stress was retarded in both the genotypes at all stages of observation i.e. 3, 5, and 7th day in all the cases. The reduction in root growth under water stress condition was greater in variety Samrat than long root mutant (Fig.3). At 7th day, the root growth of long root mutant was reduced from 7.83 cm (0 bars) to 5.17 cm (-7.5 bars), while for Samrat the reduction was from 4.30 cm (0 bars) to 1.83 cm (-7.5 bars). Better root growth in long root mutant at low water potential as compared to Samrat suggested that the development of strong root system in long root mutant right from seedling stage might have helped to draw comparatively more water to survive under water limiting situation. When most of the moisture from the upper layer is exhausted, then plant is unable to extract water to satisfy the evapotranspiration demand even though soil water available in the deeper layer is still high. Under these circumstances, deeper root may have an advantage (Passioura, 1981). The number of roots per plant was decreased with decrease in water potential but no significant differences were noticed between long root mutant and Samrat at 7th day. The shoot length was little affected in long root mutant as compared to Samrat at lowest water potential (-7.5 bars). Reduction in root and shoot growth in response to decreasing external water potential was probably due to lower water uptake, cell expansion and cell division (Zhu, 2001). A significant reduction in seedling growth in response to moisture stress was confirmed earlier results (Singh and Afria, 1985; Dutta and Bera, 2007). The results in the field experiment are also important to confirm and support the results obtained in laboratory conditions. Hence, the field experiment was also conducted for comparison of long root mutant and its parent variety Samrat under simulated water stress condition. Significant differences were noticed between long root mutant and its parent variety for all seven traits. Long root mutant recorded significantly greater mean root length (24.34 cm), number of pods per plant (19.72), seed yield per plant (4.91g),100 seed weight (4.05 g) and root length: shoot length ratio (0.70) as compared to Samrat of which mean root length (15.2 cm), number of pods per plant (13.5), seed yield per plant (3.38 g), 100 seed weight (3.72 g) and root length: shoot length ratio (0.42) were less under simulated moisture stress condition (Table 3). After considering the results, long root mutant found to perform better under osmotic and moisture stress in soil than its parent Samrat. As there are no other morphological differences in long root mutant and variety Samrat except for root length, the results clearly indicated the contribution of long root character to moisture stress. The better root length of long root mutant (LRM) under PEG induced water stress as well as simulated water stress in field conditions indicated that it might be lead to develop a strong root system which will help the genotype to draw comparatively more water to survive and reproduce normally under water scarcity situation.

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Table: 1. Effect of PEG-6000 induced moisture stress on water uptake and germination per cent in Long Root Mutant and its parent variety Samrat in mungbean

Genotype	PEG conc. (bars)	Weight of 100 seeds in grams (% water uptake)			Germination %
		After 12 hours	After 24 hours	After 36 hours	
Samrat	0 (control)	10.37 (107.4)	12.8 (156.0)	19.19 (283.8)	100
	-2.5 (15%)	8.39(67.8)	10.19(103.8)	18.68(273.6)	100
	-5.0 (20%)	7.62(52.4)	10.06(101.2)	12.74(154.8)	100
	-7.5 (25%)	7.12(42.4)	9.75(95.0)	11.89(137.8)	100
	-11.0 (30%)	6.48(29.6)	9.28(85.6)	9.78(95.5)	70
	-15.0 (35%)	5.78(15.7)	7.66(53.2)	8.10(62.0)	-
Long root mutant	0 (control)	11.14(122.8)	13.84(176.7)	20.84(316.8)	100
	-2.5 (15%)	9.94(98.8)	13.28(165.6)	20.55(311.0)	100
	-5.0 (20%)	7.81(56.2)	10.85(117.0)	16.48(229.6)	100
	-7.5 (25%)	7.76(55.2)	10.43(108.6)	15.95(219.0)	100
	-11.0 (30%)	7.41(48.2)	10.10(102.0)	10.88(117.6)	100
	-15.0 (35%)	5.96(19.2)	8.42(68.4)	8.58(71.6)	-
Genotype	S.E.±	0.029	0.111	0.084	
	C.D. (5%)	0.083	NS	0.317	
Treatment	S.E.±	0.051	0.192	0.146	
	C.D. (5%)	0.144	0.723	0.549	
Interaction	S.E.±	0.071	0.272	0.207	
(G x T)	C.D. (5%)	0.203	1.023	0.778	

* Values in parentheses are per cent water uptake

Table: 2. Effect of PEG-6000 induced moisture stress on root length, shoot length and number of roots in Long Root Mutant and its parent variety Samrat in mungbean

Genotype	PEG conc. (bars)	Root length (cm)			Number of roots at 7th day	Shoot length (cm) at 7th day
		3rd day	5th day	7th day		
Samrat	0 (control)	1.13	2.86	4.30	18.20	4.24
	-2.5 (15%)	0.77	2.59	3.57	14.14	4.15
	-5.0 (20%)	0.48	1.90	3.13	12.33	3.48
	-7.5 (25%)	0.27	1.34	1.83	11.36	2.03
Long root mutant	0 (control)	1.80	3.79	7.83	23.68	5.23
	-2.5 (15%)	1.28	3.57	6.50	19.65	5.04
	-5.0 (20%)	0.75	2.62	5.33	12.34	4.80
	-7.5 (25%)	0.34	2.03	5.17	10.28	4.21
Genotype	S.E.±	0.039	0.057	0.189	0.70	0.083
	C.D. (5%)	0.118	0.370	1.234	NS	0.438
Treatment	S.E.±	0.055	0.081	0.268	0.99	0.117
	C.D. (5%)	0.260	0.382	1.268	4.72	0.762
Interaction	S.E.±	0.078	0.11	0.379	1.41	0.165
(G x T)	C.D. (5%)	0.369	0.540	1.793	6.67	0.783

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Table: 3. Comparison of Long Root Mutant and its parent variety Samrat under simulated water stress in field conditions for grain yield and yield contributing characters in mungbean

Genotypes	Mean values						
	Root length (cm)	Shoot length (cm)	Number of branches /plant	Number of pods/plant	100 seed weight (g)	Seed yield /plant (g)	Root : Shoot ratio
Samrat	15.2	36.95*	2.0*	13.5	3.72	3.38	0.42
Long root mutant	24.35*	35.49	1.89	20.05*	4.05*	4.91*	0.69*
$t_{cal.}$	192.02	21.79	9.73	48.91	56.17	43.34	195.23
$t_{5\%\ table}$				1.96			

*Significant at $n_1 + n_2 - 2 = 65$ d.f., where $n_1=10$ and $n_2 = 57$

Fig.1: Long root mutant and its parent Samrat

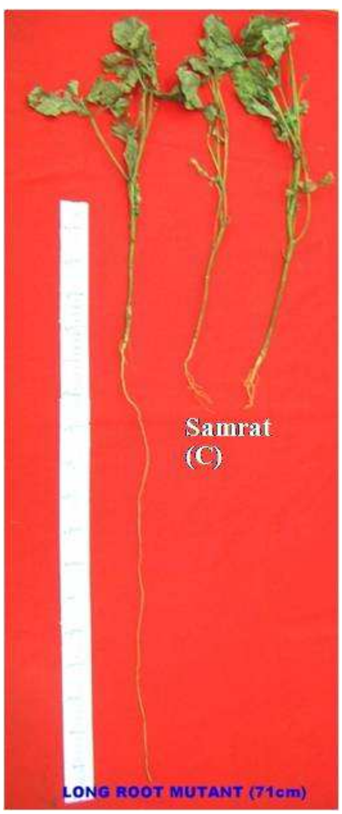

Fig.2: Effect of stress on germination and radical growth of Long root mutant and its parent Samrat (3rd day)

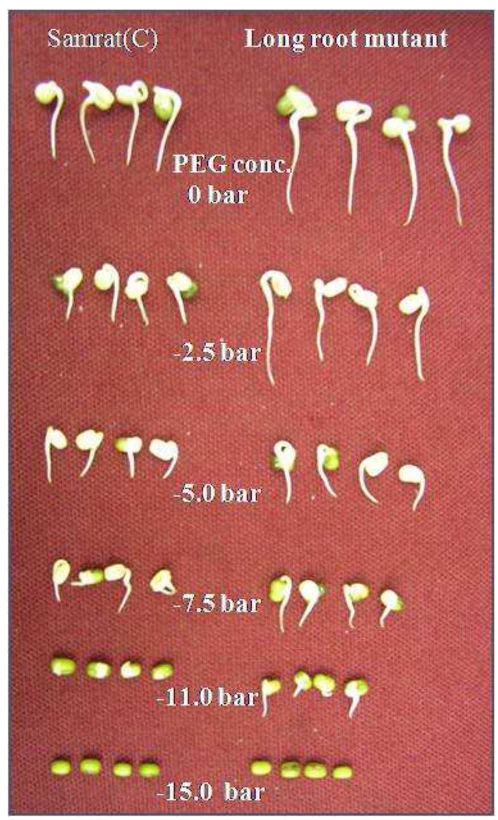

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Fig.3: Effect of stress on shoot and root growth of Long root mutant and its parent Samrat (7th day)