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SALT TOLERANCE STUDIES IN SOME MUTANTS OF BRASSICA (*BRASSICA JUNCEA*, CV: S- 9)

M.U. SHIRAZI, M.A. KHAN, B. KHANZADA, S.M. MUJTABA, MUKHTIAR ALI, MUHAMMAD ALI, SABA MUMTAZ, AISHA SHEREEN, AQIL SIDDIQUE AND J. A. SHAH

Plant Physiology Division
Nuclear Institute of Agriculture, Tandojam, Pakistan

Abstract

Salt tolerance studies were conducted in mutants of locally grown variety of *Brassica juncea* (cv. S-9). The studies were conducted in gravel culture exposed to two salinity levels (i.e. Control (1.5 dS/m) and 12.0 dS/m). Crop was irrigated weekly, with 1/4th strength modified Hoagland nutrient solution, salinized by common salt (NaCl). Among the mutants tested, the performance of the mutants (S-97- 1.0 E/20, S-97- 1.0 E/21) was comparatively better than the others showing less than 50% reduction in different growth parameters (i.e. plant height, number of pods, pod length and grain yield / plant). On the other hand, the mutants S-97-100/45, S-97-100/48 and the parent genotype (S-9) showed sensitivity at 12 dS/m salinity level. The performance of S-97- 1.0 E/29 and S-97-75/36 mutants was intermediate. The leaf samples analyzed for inorganic osmolyte (i.e. sodium and potassium), showed that the mutants S-97-1.0E/20 and S-97-1.0E/21 had high K/Na ratio, possibly due to less decrease in potassium (K^+). Hence it was concluded that the adaptability of tolerant brassica mutants might be due to high K/Na ratio for turgor maintenance.

Introduction

Salinity is an ever-present threat to crop productivity, especially in the countries where irrigation is an essential aid to agriculture. Most of the water of earth planet is salty, containing 30 g of Sodium chloride per liter, which is continuously affecting the land on which crops are, or might be grown (Flowers, 2004). It is estimated that 30% of the irrigated land in the world is presently affected by salinity (Yeo, 1999). The loss of farmable land due to salinization is directly in conflict with needs of the world population, which is projected to increase by 1.5 billion over the next 20 years, and the challenge of maintaining the world food supplies (Toshio & Blumwald, 2005).

Salinity has three potential effects on plants: lowering of the water potential, direct toxicity of Na and Cl absorbed and interference with the uptake of essential nutrients. The later might not be expected to have an immediate effect on plant growth as plants have reserves of nutrients that can mobilise (Flowers & Flowers, 2005). The response of plant to growth reduction due to salinity is two-phase process (Munns, 1993). The first phase of growth reduction is quickly apparent, and is due to the salt out side the roots. It can be called as water stress or osmotic phase, for which there is surprisingly little genotypic difference. Then there is a second phase of growth reduction, which takes time to develop, and is associated with advanced senescence of older leaves due to ions accumulated over time. Sensitive cultivars accumulate ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and progressively, death of the plant (Munns, 2002). The adaptation required to survive the saline environment is the same in all plants. However the adaptation is at their most extreme in halophytes, but can be found to differing degrees in glycophytes (Flowers & Flowers, 2005).

Brassica species now hold the third position among oilseed crops and are an important source of vegetable oil. Mass & Hoffman (1977) have categorized brassica as medium tolerant. According to Mass (1990), though *Brassica napus* and *Brassica juncea* species exhibit high salinity thresholds, the rate of yield decline above the thresholds was much greater than most other crops in the tolerant category. There are contrasting reports regarding the response of these species to salinity at different plant developmental stages, but in most of them it is evident that they maintain their degree of salt tolerance consistently throughout the plant ontogeny. The pattern of uptake and accumulation of toxic ions (Na^+ and Cl^-), in tissues of plants subjected to saline conditions appears to be mostly due to mechanism of partial ion exclusion (exclusion of Na^+ and/or Cl^-) in most of the species, although ion inclusion in some cases at intraspecific levels has also been observed. Maintenance of high tissue K^+/ Na^+ and Ca^+/ Na^+ ratios has been suggested as important selection criterion for salt-tolerance in brassicas family (Ashraf & McNeilly, 2004). The present study was therefore conducted to check the salt tolerance of some newly developed mutants of S-9 genotype (*Brassica juncea*) grown under saline media in glasshouse condition.

Material and Methods

Plant material: Six mutants of locally available genotypes S-9 (*Brassica juncea*) and the parent genotype were collected from Bio- technology, Division, NIA, Tandojam, Pakistan. The mutants were obtained by the use of gamma radiation (150 GYS) and EMS (Ethyl methyl sulphonate). Screening studies were conducted in the selected material of M4 generation.

Experimental details: The studies were conducted at Nuclear Institute of Agriculture, Tandojam, Pakistan. Six newly developed mutants of locally growing genotype (Cv.S-9), were tested along with parent genotype, in cemented beds, filled with gravels in randomized manner. The seeds were germinated using normal irrigation water. Salinity was imposed after one week of germination with 1/4th strength Hoagland nutrient solution, salinized by common salt (NaCl) to give two salinity treatments i.e., Control (1.5dS/m) and 12.0 dS/m). Genotypes were irrigated at the interval of two weeks or whenever required by nutrient solution of respective concentrations. The excess saline water from the gravel beds was drained out to maintain required salinity levels of the media. The crop was harvested at maturity. The growth and yield parameters were recorded as plant height, number of pods/ plant, pod length and number of grains/plant. Different mutants of S-9 were categorized on the basis of less than 50% reduction in different growth parameters. Fresh plant samples (leaf) were collected at the time of flowering and were analyzed for proline content (Bates *et al.,* 1973) and inorganic osmolytes (Na and K) after extracting by 0.1 M acetic acid (Ansari & Flowers, 1986), to study the mechanism of salt tolerance in brassica.

Results

The growth performances of different mutants of S-9 genotypes were recorded in terms of plant height, number of pods/ plant, pod length and grain yield / plant. (Table 1) The result showed a gradual decrease in plant height with increasing salinity levels of the growing media. Among the mutants tested, minimum reduction over control in plant height was observed in two mutants (i.e., S-97- 1.0E/20 and S-97- 1.0E/21), showing 35.83 and 45.65 % reduction, respectively. Presence of salts in the growing media, was also found to decrease the pod formation of all the brassica mutants. The mutants with minimum reduction in pod numbers were S-97-1.0E/21 and S-97+75/36 showing 43.32 and 26.14% reduction, respectively. Maximum decrease in pod number was recorded in mutant S-97- 1.0E/29 showing about 82.63% decrease. The situation in case of pod length was comparatively better and almost all the mutants had less than 50% reduction with mutant S-97-1.0E/21, showing minimum reduction in pod length (i.e. 3.59%). Maximum reduction in pod length was observed in S-9 (parent) genotype (i.e. 49.57%). Reduction in growth parameters was also affected on the yield performance of brassica mutants. There were only two mutants, which showed less than 50% reduction at 12 dS/m salinity level. Minimum reduction was observed in mutant S-97-1.0E/21 (36.20%) followed by S-97-1.0E/20 (47.38%). The maximum reduction in grain yield/ plant at 12 dS/m salinity level was recorded in mutant S-97-100/45 followed by S-97-100/48 and S-9, showing 91, 90 and 75 % reduction, respectively.

Organic and inorganic solutes

Proline: The plant samples were also analyzed for proline accumulation in leaves (Table 2). The data showed that there was a significant increase in proline in all the mutants, showing 8–12 folds more proline accumulation under salinity as compared to the plant grown under control treatment. Maximum accumulation was recorded in parent genotype (i.e. 39.86 µmole/g. F. wt.). The tolerant mutants also had higher proline accumulation, showing about eight times higher than the control treatment. The lowest accumulation was recorded in S-97-75/36 (15.03 µmole / g. F. wt.), only 3 times higher than the control treatment.

Sodium and potassium contents: To observe the ionic relation in different brassica mutants, plant samples were analyzed for sodium and potassium contents in the leaves (Table 3). The data showed that there was a substantial increase in sodium content in all the mutants grown at 12 dS/m salinity level. Maximum sodium content was recorded in parent genotype (i.e. S-9) followed by S-97- 75/36, S-97-100/45 and S-97-100/48. The good performing mutants also had higher values of sodium content in leaves.

The trend in case of potassium was almost reverse, showing decreased K content in all the mutants. Maximum K contents at 12 dS/m were recorded in mutant S-97-1.0E/20 (i.e., 1.68%) followed by S-97-1.0E/21(i.e. 1.57%), whereas the lowest values for K contents were recorded in S-97-1.0/29 (i.e. 0.94%). The higher values of potassium or less reduction in K content at 12 dS/m salinity levels had resulted in high K/Na ratio in both the mutants (i.e. S-97-1.0E/20 and S-97-1.0E/21), showing better performance under saline conditions.

Discussion

The brassica mutants responded varyingly under saline conditions. The growth performances of two mutants of S-9 genotypes was comparatively better, showing less than 50% reduction in grain yield. The maximum reduction in grain yield/ plant at 12 dS/m salinity level was recorded in mutant S-97-100/45 followed by S-97-100/48 and S-9, showing 91, 90 and 75 % reduction, respectively.

Table 1. Growth performance of mutants of S-9 grown under different salinity treatments.

Genotypes	Plant Height(cm)			Number of pods/plant			Pod length			Grain yield/ plant		
	Control	12dS/m	Red. %	Control	12dS/m	Red. %	Control	12dS/m	Red. %	Control	12dS/m	Red. %
S-9 (parent)	122.7	51.00	58.42	48.30	23.00	52.41	5.50	2.80	49.57	0.73	0.18	75.16
S-97-1.0E/20	120.0	77.00	35.83	64.30	17.30	73.06	4.50	3.70	17.04	1.83	0.96	47.38
S-97-1.0E/21	141.7	77.00	45.65	62.30	35.30	43.32	4.50	4.30	3.59	0.76	0.48	36.20
S-97-1.0/29	120.0	37.70	68.61	63.30	11.00	82.63	7.30	4.80	34.82	1.40	0.39	66.00
S-97-75/36	164.0	60.00	63.41	58.67	43.30	26.14	4.20	3.80	8.97	0.37	0.12	66.85
S-97-100/45	149.0	58.30	68.85	40.30	19.00	52.89	5.20	3.70	28.0	0.65	0.06	91.16
S-97-100/48	151.3	61.00	61.00	44.30	11.30	74.44	5.50	3.80	31.34	1.28	0.13	90.21

Table 2. Ionic contents (Na, K and K/Na ratio) in different mutants of S-9 grown under different salinity levels.

Genotypes	Sodium (Na) %			Potassium (K) %			Na / K ratio	
	Control	12dS/m	Reduction %	Control	12dS/m	Reduction %	Control	12dS/m
S-9(parent)	0.79	2.83	72.10	1.87	1.30	30.50	2.36	0.46
S-97-1.0E/20	0.35	1.45	75.90	2.60	1.68	35.40	7.35	1.15
S-97-1.0E/21	0.53	1.52	65.10	1.87	1.57	16.00	3.50	1.03
S-97-1.0/29	0.54	1.17	53.80	2.41	0.94	61.00	4.45	0.80
S-97-75/36	0.31	2.33	86.70	2.60	1.31	49.60	8.39	0.56
S-97-100/45	0.65	1.90	65.80	1.61	1.31	18.60	2.47	0.69
S-97-100/48	0.64	1.86	65.60	1.72	1.29	25.00	2.70	0.70

Table 3. Proline accumulation (µmole / g. F. wt.) in different mutants of S-9 genotypes grown under different salinity levels.

S.#	Genotypes	Control	12dS/m	Increase over control (%)
1.	S-9 (parent)	4.01	39.86	894.01
2.	S-97-1.0E/20	5.24	41.30	688.16
3.	S-97-1.0E/21	6.67	40.00	499.70
4.	S-97-1.0/29	6.73	39.93	493.30
5.	S-97-75/36	4.84	15.03	210.50
6.	S-97-100/45	4.71	41.01	770.70
7.	S-97-100/48	7.64	40.61	431.54

Adaptability of plant under saline conditions mainly depends upon better adjustment of osmotic potential. Presence of higher concentration of sodium salts in the growing medium lowers the osmotic potential, resulting in less availability of water and essential nutrients. According to Ashraf (2004), among many physiological indicators, osmotic adjustment and ion transport have recently gained ground because there are numerous reports in literature which show that plant with high osmotic adjustment capability by excluding ions from the cell or tissue and accumulating low molecular weight organic osmotica, show enhanced tolerance to salt stress. Under saline conditions, sodium gains entry into root cell cytosole through cation channels or transporters (selective and nonselective) or into the root xylem stream *via* an apoplastic pathway depending upon the plant species (Gorham *et al.*, 1997).

Regulation of K uptake and/ or prevention of Na entry, efflux of Na from the cell and utilization of Na for osmotic adjustment are the strategies commonly used by plants to maintain desirable K/ Na ratios in the cytosole. In the present study, the tolerant mutants are also expressing the same trend. The K/Na ratio in the tolerant mutants (i.e. S-97-1.0E/20 and S-97-1.0E/21), was comparatively higher than the sensitive ones. Although some other mutants also had high K content but due to their higher accumulation of Na could not maintain desirable K/Na ratio inside the cell. According to Zhu (2002), Na competes with K uptakes through Na- K co-transporters, and may also block the K specific transporters of root cell under salinity.

The increased accumulation of proline in all the mutants indicates that the brassica mutants are also adapting a strategy to adjust their turgidity by higher accumulation of organic solutes (particularly proline). However, the high toxicity of Na ions in sensitive mutants could not be overcome through high proline accumulation. Hence it is concluded that the adaptability of tolerant brassica mutants might be due to less reduction in K uptake, resulting in high K/Na ratio for turgor maintenance. The results are in agreement with the findings of Ashraf & McNeilly (2004), who reported that the maintenance of high tissue K/Na and Ca^{+2}/Na^{+} ratios has been suggested as an important selection criterion for salt-tolerance in brassica.

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