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Research Article

Induced drought and salinity tolerance in the variants of *Brassica campestris* (L.).

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Abstract: The variants obtained from M_2 generation of gamma irradiated toria varieties, T-9 and PT303 of *Brassica campestris* (L.) were evaluated for drought and salt tolerance. Among them, variant TV4 of var. T-9 and the variants PD3 and PD4 of PT303 showed significantly higher antioxidant enzyme activities, proline content, relative water content percentage and yield per plant under these stress conditions. These variants appear more adaptive which may be assigned to their induced favorable genotypic alterations, and could be utilized for developing drought and salinity tolerant *Brassicas*.

Keywords: *Brassica campestris* (L.), drought, salinity, stress, tolerance, variants.

Introduction

Brassica campestris (L.) is an important edible oil yielding crop and occupies second position in the world in oilseed production. In developing countries their production lags behind than its present requirement due to several environmental stresses and non-availability of desirable improved genotypes. Among them drought and salinity are the major abiotic stresses conflicting the crop productivity in arid and semi arid regions. These stresses negatively affect many physiochemical processes of plants leading to accumulation of ROS and reduction in growth and yield. Screening of desirable genetic variability is prerequisite for developing drought and salt resistant genotypes in *Brassica*. The genetic variability may be induced and exploited through mutagenesis.

Plant adopts to stress by different mechanisms including changes in various morphological and physiological processes (Munns 2000, Zhou 2001). Hence assessment of these processes is an important criterion for the selection of drought and salt tolerant genotypes with better capacity to grow under drought and salt affected soil, and leading to increased net cropped area which may consequently lead to enhanced production.

Thus improvement and evaluation of certain superior *Brassica* genotypes with enhanced protection from oxidative damage and better yield even in stress condition is extremely important. Hence drought and salt tolerance potential of gamma rays induced eight variants of T-9 and PT303 var. of *Brassica campestris* (L.) was undertaken by physiological, biochemical and yield studies.

Materials and Methods

The seeds of eight variants obtained from M_2 generation of gamma irradiated toria vars. PT303 and T-9 of *Brassica campestris* (L.) and their parents (Table-1).

Table1. Traits of variants and parents of two *Brassica campestris* (L.) varieties.

Genotypes	Characters	Genotypes	Characters
T9	Parent	PT303	Parent
TV1	4-6 Petals	PD1	Tall and vigour
TV2	Tall with short pod	PD2	Dwarf, bold and bright seeded
TV3	Vigorous and high yielding	PD3	High yielding with long pod
TV4	Brown and bold seeded	PD3	Bold seeds, semi dwarf

These seeds were sown in sterilized sand containing PVC pot in glass house condition. Germinated seedlings were then supplied by half strength Hoagland nutrient solution (Hewitt 1968) until plant developed 4-6 expanded leaves. Thereafter, the pots were divided into three lots, one for control and other two for drought and salt treatment. Drought and salinity were created by half strength Hoagland nutrient supplemented with 1 %(w/v) Polyethylene glycol and 1% (w/v) common salt (NaCl) respectively. The plants were supplied with nutrient solution in respective concentrations regularly. After 10 days of treatment when stress symptoms were obvious, the samples of fresh leaves were collected for estimating antioxidant enzymes activities, total protein, proline content and RWC percentage. At maturity the crop was harvested for total seed yield. Activities of antioxidant enzymes viz. peroxidase and catalase were estimated in leaf tissues according to the method of Luck (1973) and

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Euller and Josephson (1973) respectively. For expressing specific activity, soluble protein contents in the extract was estimated by methods of Lowry *et al.,* (1983). Proline content was estimated as per Bates *et al.,* (1983) and Relative water content percentage was estimated according to Barr and Weatherly (1962).

Statistical analysis

Triplicate data were summarized as Mean ± SE. Groups were compared by two factor (Treatment and Variety) analysis of variance (ANOVA) and the significance of mean difference between groups were done by Newman-Keuls post hoc test. A two-tailed (α=2) probability $p<0.05$ was considered to be statistically significant. STATISTICA version 6.0 was used for the analysis.

Results and Discussion

The activities of peroxidase (EC 1.11.1.7) and catalase (EC.1.11.1.6) were found significantly enhanced in the induced variants and their parents on treatment with 1% Polyethylene glycol and 1% NaCl. Similar responses were reported in sunflower (Zhang 1996); rice (Shrivalli *et al.,* 2003) under drought and in wheat under salt stress (Sairam *et al.,* 2002). However, significantly higher activities of these antioxidant enzymes were recorded in variant TV4 under both the stress conditions and also it was true for PD4 in drought and PD3 under salt treatment (Table 2, 3). Our results are in consonance with earlier reports that stimulation in the activities of these enzymes were more pronounced in resistant varieties under stress conditions (Gosset *et al.,* 1994, Kraus *et al.,* 1995, Mittova *et al.,* 2004, Asharaf *et al.,* 1990) and it was suggested that the enhancement of antioxidant system is closely related to drought and salt tolerance of the crops.

The proline accumulation was remarkably increased in all the variants under both the treatments and was higher in salt treatment. The variants, TV4, PD3 and PD4 had significantly higher proline accumulation (Table 2 and 3). Increased proline content has earlier been reported in mustard (Alia *et al.,* 1993), wheat (Singh *et al.,* 1983) and sesame (Koca *et al.,* 2007). The genotypic differences in proline accumulation under stress were used as an index for determination of tolerance among the cultivars (Mishra *et al.,* 2005). High proline content plays an important role in cell osmoregulation and protects plants from dehydration under adverse conditions (Saleik *et al.,* 1993).

Relative water content percentage was considerably decreased in all the variants under drought and salt stress. However, among the variants and parents the least significant decrease was also found in TV4, PD4 and PD3 (Table 2 and 3). Similar findings have also been reported in *Brassica* (Madan *et al.,* 1994), rice (Sairam *et al.,* 2004) under stress condition. The high RWC% is to be associated with the high proline content which helps in osmotic adjustment and balances the leaf water status (Aspinal *et al.,* 1981). The greater retention of RWC% in these variants indicated greater tolerance capacity to withstand drought and salt stress (water deficit conditions).

The yield performance of the variants varied under both the stress conditions. Our results show that the variants TV4, PD4 and PD3 are superior in respect of yield, antioxidant defense system as well as proline content and RWC%, and could be relatively more adaptive under stress conditions.

Conclusion

The higher yield and enhancement in antioxidant enzyme activity of variants under both the stresses may be due to induced favorable genotypic alterations by gamma irradiation. These variants can be exploited for developing drought and salinity tolerant *Brassicas.*

Table 2: Stress parameters (Mean ± SE, n=3) of different variants of T-9 at three different treatments;

Parameters	Variety	Treatments		
		Control	Drought	Salinity
Catalase (H_2O_2 split/mg protein)	T-9	317.00 ± 6.08	472.67 ± 6.39^{c}	392.00 ± 6.43^{cd}
	TV1	520.33 ± 6.06^{a}	406.00 ± 5.51^{ac}	374.67 ± 5.55^{cd}
	TV2	452.67 ± 5.46^{a}	395.00 ± 6.43^{ac}	390.00 ± 6.08^{c}
	TV3	394.00 ± 6.03^{a}	416.67 ± 5.61^{a}	413.67 ± 5.78
	TV4	432.33 ± 5.78^{a}	620.00 ± 5.86^{ac}	562.00 ± 6.43^{acd}
Peroxidase (act./mg protein)	T-9	2.51 ± 0.10	2.58 ± 0.07	2.63 ± 0.09
	TV1	2.27 ± 0.09	2.53 ± 0.09	2.27 ± 0.09
	TV2	1.79 ± 0.07^{a}	1.66 ± 0.08^{a}	2.02 ± 0.10^{ad}
	TV3	1.74 ± 0.06^{a}	1.82 ± 0.10^{a}	2.31± 0.07^{cd}
	TV4	2.47 ± 0.08	3.08 ± 0.07^{ac}	3.25 ± 0.10^{ac}
Proline (µmol/mg f.wt.)	T-9	42.27 ± 0.61	52.67 ± 0.88^{c}	85.30 ± 0.85^{cd}
	TV1	37.25 ± 0.72^{a}	40.20 ± 0.76^{ac}	82.17 ± 0.73^{acd}
	TV2	33.23 ± 0.96^{a}	40.37 ± 0.73^{ac}	76.13 ± 0.70^{acd}
	TV3	30.17 ± 0.73^{a}	31.40 ± 0.70^{a}	77.00 ± 0.75^{acd}
	TV4	23.33 ± 0.71^{a}	44.23 ± 0.96^{ac}	95.27 ± 0.93^{acd}
RWC (%)	T-9	84.87 ± 0.81	84.33 ± 0.88	81.00 ± 0.58^{c}
	TV1	86.00 ± 1.00	83.20 ± 0.76	71.40 ± 0.70^{acd}
	TV2	85.33 ± 0.88	83.00 ± 1.00	72.20 ± 0.64^{acd}
	TV3	89.00 ± 0.58^{a}	85.17 ± 0.73^{c}	76.17 ± 0.66^{acd}

	TV4	88.17 ± 1.01	87.50 ± 0.81	82.43 ± 0.98^{cd}
Seed yield (g/pl)	T-9	0.63 ± 0.02	0.48 ± 0.01^{c}	0.35± 0.01^{cd}
	TV1	0.69 ± 0.01	0.60 ± 0.01^{ac}	0.40 ± 0.02^{cd}
	TV2	0.71 ± 0.03	0.54 ± 0.02^{c}	0.22 ± 0.01^{acd}
	TV3	1.32 ± 0.04^{a}	0.70 ± 0.03^{ac}	0.39 ± 0.02^{cd}
	TV4	0.80 ± 0.03^{a}	0.75 ± 0.03^{a}	0.47 ± 0.03^{acd}

$^{a}p<0.05$ or $^{a}p<0.01$ in comparison with Parent (T-9)
$^{c}p<0.05$ or $^{c}p<0.01$ in comparison with Control
$^{d}p<0.05$ or $^{d}p<0.01$ in comparison with Drought

Table3. Stress parameters (Mean ± SE, n=3) of different variants of PT303 at three different treatments;

Parameters	**Variety**	**Treatments**		
		Control	**Drought**	**Salinity**
Catalase (H_2O_2 split/mg protein)	PT303	506.00 ± 6.08	493.00 ± 5.69	534.00 ± 5.86^{cd}
	PD1	409.67 ± 6.49^{a}	527.67 ± 5.46^{ac}	477.00 ± 5.69^{acd}
	PD2	600.33 ± 6.57^{a}	532.00 ± 6.35^{ac}	428.33 ± 5.84^{acd}
	PD3	520.00 ± 6.24	492.00 ± 6.43^{c}	681.67 ± 6.01^{acd}
	PD4	611.00 ± 6.51^{a}	668.33 ± 6.57^{ac}	462.00 ± 6.11^{acd}
Peroxidase (act./mg protein)	PT303	1.77 ± 0.02	1.53 ± 0.04^{c}	1.90 ± 0.02^{d}
	PD1	1.67 ± 0.03	1.34 ± 0.03^{ac}	1.87 ± 0.03^{cd}
	PD2	1.62 ± 0.05^{a}	1.51 ± 0.05	1.76 ± 0.04^{cd}
	PD3	1.47 ± 0.03^{a}	1.37 ± 0.03^{a}	2.03 ± 0.05^{acd}
	PD4	1.11 ± 0.02^{a}	1.83 ± 0.04^{ac}	1.77 ± 0.04^{c}
Proline (μmol/mg f.wt.)	PT303	14.80 ± 0.40	32.53 ± 0.26^{c}	81.53 ± 0.38^{cd}
	PD1	15.48 ± 0.31	20.23 ± 0.50^{ac}	78.80 ± 0.42^{acd}
	PD2	14.90 ± 0.21	20.87 ± 0.47^{ac}	62.13 ± 0.47^{acd}
	PD3	14.48 ± 0.37	19.83 ± 0.44^{ac}	83.40 ± 0.31^{acd}
	PD4	19.53 ± 0.59^{a}	70.65 ± 0.46^{ac}	70.50 ± 0.29^{ac}
RWC (%)	PT303	89.13 ± 0.70	85.07 ± 0.64^{c}	69.80 ± 0.74^{cd}
	PD1	89.53 ± 0.74	87.40 ± 0.83	74.77 ± 0.79^{acd}
	PD2	85.07 ± 0.64^{a}	87.90 ± 0.86	78.00 ± 0.81^{acd}
	PD3	85.90 ± 0.67^{a}	82.93 ± 0.69	79.67 ± 0.88^{acd}
	PD4	83.82 ± 0.66^{a}	83.13 ± 0.70	78.53 ± 0.74^{acd}
Yield (g/pl)	PT303	0.72 ± 0.02	0.58 ± 0.04^{c}	0.23 ± 0.03^{cd}
	PD1	0.92 ± 0.04^{a}	0.56 ± 0.03^{c}	0.38 ± 0.02^{acd}
	PD2	0.85 ± 0.05^{a}	0.52 ± 0.04^{c}	0.14 ± 0.03^{cd}
	PD3	1.03 ± 0.05^{a}	0.84 ± 0.03^{ac}	0.56 ± 0.02^{acd}
	PD4	1.26 ± 0.04^{a}	1.10 ± 0.05^{ac}	0.20 ± 0.03^{cd}

$^{a}p<0.05$ or $^{a}p<0.01$ in comparison with Parent(PT303)
$^{c}p<0.05$ or $^{c}p<0.01$ in comparison with Control
$^{d}p<0.05$ or $^{d}p<0.01$ in comparison with Drought

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