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Reproductive Behaviour of *Brassica juncea* as Exposed to Drought Stress at Different Days after Sowing in Jammu Region

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A B S T R A C T

The drought tolerance of *Brassica juncea* genotypes *viz*., Kranti RSPR-03, RSPR-01, Pusa Bold and NRCDR-2 was investigated after exposure to at various growth stages in a pot experiment. Water stress imposed at branch initiation, flower initiation and siliquae formation stages in the form of 45, 60 and 90 (DAS) days after sowing. Various physiological and reproductive behavior were recorded which revealed significant differences among the various *Brassica juncea* genotypes. The drought susceptibility index (DSI) for seed yield was recorded to characterize the relative tolerance of genotypes. Relative water content was reduced maximum under drought stress conditions. Reduction in seed yield was observed when water stress induced at flowering stage and it also affects siliquae development. It was noticed that reduction in seed weight was minimum when water stress induced before flowering*i.e*., at 60 days after sowing. Average yield was found greater in Kranti and least in Pusa Bold. The findings of the present research investigation recommended the growing of Kranti in the drought prone areas to obtain high economic yield even in adverse condition.

Introduction

Water deficit stress has effect on vegetative and reproductive stages of canola. The effect of water deficit stress was more during reproductive growth than vegetative growth of rapeseed (Ghobadi *et al.*, 2006). The effect of drought stress is a function of genotype, intensity and duration of stress, weather conditions, growth, and developmental stages of rapeseed (Robertson and Holland, 2004). The water deficiency can influence inversely the grain of canola but this effect depends on the genotype, growth stage and the plant adaptation to the drought (Azizi *et al.*, 1999). The water deficiency has the greatest effect on the grain yield of canola in flowering and pollination stage.

Therefore, study of different traits including relative yield of genotypes under stressed and non-stressed conditions is as a starting point for understanding the drought tolerance process and selection of genotypes to improve in dry regions (Fernandes, 1992). The yield and biochemical composition of a plant mainly depends on growth conditions, which is markedly affected by water availability (Sakova *et al*., 1995. Paclik *et al*., 1996). The most pronounced effects are observed when the water shortage occurs during the flowering period or pod-filling stages. At reproductive phase, water stress accelerates the process of flower and fruit drop and decreased seed yield (Gan, *et al*., 2004, Sinaki, *et al*., 2007). All these factors contribute to reduced dry matter accumulation and grain yield under drought. Drought strongly affects crop phenology by shortening the crop growth cycle with a few exceptions. Limited water supply triggers a signal to cause an early switching of plant development from the vegetative to reproductive phase (Desclaux and Roumet, 1996).

It has been recognized that plants exhibit several adaptations to survive under stress conditions. Reduced leaf area, stomatal closure to prevent the transpirational water loss, decreased stomatal conductance, limited internal CO_2 concentration, reduced photosynthesis are very vital (Chaves *et al.*, 2009). These responses in turn trigger the cellular responses viz., diminished leaf water potential, loss of turgor, changes in solute concentration and osmotic adjustment (Morgan and King, 1984). Water-deficit stress tolerance is thus the result of coordination of physiological and biochemical alterations at the organ, cellular and molecular levels. Generally, plants respond to water deficit stress through developmental, biochemical and physiological changes and the type of the observed response depends on several factors such as stress intensity (SI), stress duration and genotype (Moradshahi *et al*., 2004).

In order to overcome these problems, genotypes which are tolerant to drought stress are to be identified. Selection and breeding programme to increase high temperature tolerance was more successful and the selection was based directly on the physiological mechanism(s) or character (s) conferring tolerance.It would therefore, be important to identify the morpho-physiological and biochemical traits for drought stress tolerance at the reproductive stage because this stage is much important in terms of economic yield.

Materials and Methods

Plant material and growth condition

Five *Brassicajuncea* genotypes viz., Kranti RSPR-03, RSPR-01, Pusa Bold and NRCDR-2 were used in this study. The plants were grown in pots in glasshouse. The plants were raised in earthen pots (30 cm diameter) filled with soil. Sowing was done in the last week of October.

Drought stress imposition

The plants were watered to field capacity every two days until the treatments were imposed. The plants were exposed to drought stress at three growth stages i.e. vegetative, flowering and pod filling stage. For imposing drought, water was withheld until the plants showed symptoms of wilting and leaf rolling (Sakova *et al*., 1995; Siddique *et al.*, 2000). The control plants were irrigated continuously at the optimum moisture regime. Data regarding yield and yield contributing parameters were recorded at maturity.

Experimental design and statistical analysis

Each cultivar was replicated three times in separate pots having three plants per replicate. Total 45 pots were arranged in completely randomized design with a control in each cultivar. Data were analyzed using

Completely Randomized Block Design (CRBD) for two factors. Treatments were compared using critical difference (CD) at 5 % level of significance. Data were subjected to analysis of variance (ANOVA) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar 125 004, Haryana, India).

Relative water content (RWC %) of leaf

The RWC was calculated using the formula (Weatherley, 1950).

Relative stress injury (RSI %)

The relative stress injury (RSI %) in leaves was evaluated by (Sullivan, 1972).

Reproductive parameters

Pollen viability

Viability of freshly released pollen grains was assessed by 2, 3, 5-triphenyl tetrazolium chloride (TTC) test (Hauser and Morrison, 1964).

Flowers thermo sensitivity rating/flower shedding

Average flower shedding (%) was observed from the terminal raceme on the main stem of 10 randomly selected plants. Ratings for tolerant, moderately tolerant and susceptible genotype were made as follow

Flowers shedding (%)	Rating
10-20	Tolerant
20-40	Moderate tolerant
>40	Susceptible

Days to 50% flowering

The number of days taken from planting to 50% flowering of the plants was recorded as days to 50% flowering.

Days to 50% pod formation

The number of days taken from planting to 50% pod formation of the plants was recorded as days to 50% pod formation.

Days to physiological maturity

The number of days taken from planting to harvesting of the crop or plant was recorded.

Drought susceptibility index (DSI)

A drought susceptibility index (DSI) for seed yield and its components was calculated using the formula (Fischer and Maurer, 1978)

Results and Discussion

Relative water content RWC (%) of leaf

RWC% (Table 1) of leaves in all five *B. juncea* genotypes significantly decreased from 74.71% to 38.73% with increasing the period of DAS to drought stress from control condition to 90 DAS. Maximum RWC was noticed in Kranti (59.50%) followed by RSPR-03 (55.81%) and RSPR-01(54.52%) against the minimum in Pusa Bold (47.68%) followed by NRCDR-2 (48.62%).

Relative stress injury (RSI %) of leaf

RSI% (Table 1) increased significantly with increase in DAS to drought stress in all five genotypes *i.e.* from control to 90 DAS *i.e.*,22.19% to 40.45% respectively. The maximum increase in RSI % was observed in Pusa Bold(24.31% to 47.32%) followed by NRCDR-2 (23.92% to 45.41%) and minimum was noticed in Kranti (19.95% to 32.13%). The results of RSI for genotypes and drought were statistically significant.

Reproductive studies

Pollen viability (%)

The pollen remained viable under normal conditions but the viability decreased significantly from 82.13 % to 73.28 % i.e. maximum in control followed by 45 DAS and 90 DAS as well as minimum was found in 60 DAS (Fig. 1). Genotypically Kranti showed significantly higher pollen viability (83.00 %) followed by RSPR-03 (80.41) followed by RSPR-01 (76.50 %) and minimum in NRCDR-2 (75.33 %) and Pusa Bold genotype (73.18 %) (Fig.3).

Phenological mean performance of genotypes

Flower shedding (%)

The mean flower shedding percentage was less in control (23.31) as compared to drought (35.98) at 60 DAS. The genotypes also showed significant differences for flower shedding percentage in both control and stress conditions. The mean number for flower shedding percentage of all genotypes was 23.89, 27.33, 29.02, 30.00 and 31.65 in Kranti, RSPR-03, RSPR-01, NRCDR-2 and Pusa Bold respectively (Table 1).

Days to 50% flowering

There was a significant reduction in days to 50% flowering due to stress in all five genotypes. The mean number of days to 50% flowering in control (non-stressed) was 72.7 days as compared to (stress) conditions (68.6 days) because 60 DAS was found maximum exposed to drought stress a compared to 45 and 90 DAS. Minimum number of days taken for 50% flowering was recorded in Kranti (66.5) followed by RSPR-03 (69.2) and RSPR-01 (71.3) and maximum was found in NRCDR-2 (72.5) and Pusa Bold (74.8), it means 50% flowering was slightly early in Kranti than other genotypes (Table 1).

Days to 50% pod formation

The drought stress significantly reduced the days to 50% pod formation in both conditions i.e. drought and control conditions (Table 1). The mean numbers of days to 50% pod formation were more (91.0 days) in NS (non-stressed) compared to condition (stressed) (85.3 days). The minimum days taken for 50% pod formation was in Kranti (84.6) followed by RSPR-03 (86.1) and RSPR-01 (88.1) and maximum days was taken in NRCDR-2 (90.4) as well as Pusa Bold (92.1).

Days to physiological maturity

Drought stress significantly reduced the mean maturity duration in stress conditions. The mean number of days to maturity was more in control (122.3 days) as compared to drought (116.2 days) at 60 DAS (Table 1). The genotypes also showed significant differences for days to maturity in both control and stress conditions. The mean number of days to maturity of all genotypes was 115.8,117.1, 119.1, 121.3 and 123.0 in Kranti, RSPR-03, RSPR-01, NRCDR-2 and Pusa Bold respectively.

Seed yield

The seed yield plant^{-1} of *Brassica juncea* is a cumulative effect of various yield components like no. of pods per plant, number of seed per pod and grain yield per plant. The data regarding seed yield of different *B. juncea* cultivars (Table 1) grain yield (g) was significantly affected by drought applied at 45, 60, and 90 DAS i.e., at vegetative. The maximum reduction was found at 60 DAS (3.23 g) as compared to 45 DAS (3.36 g) and 90 DAS (3.76 g).The highest grain yield were found in Kranti (4.66 g) followed by RSPR-03 (4.27 g) and RSPR-01 (3.68 g) as well as minimum yield were found in NRCDR-2 (3.29 g) and Pusa Bold (2.91 g).

Drought susceptibility index

Drought susceptibility index showed decreasing trend in all the genotypes from branch initiation stage to siliquae formation stage.The mean DSI value (Fig.4) was low in Kranti i.e. (0.37). In genotype RSPR-03 the values was 0.65 followed by RSPR-01 (0.82) and in genotype NRCDR-2 and Pusa Bold were (1.10) and (1.13) respectively.

Discussion

The leaf RWC indicates the leaf water status and is considered to be an important marker of drought tolerance in plants (Sánchez-Blanco *et al.,* 2002). Our observations are in agreement with other researchers (Loon C.D., (1981); Nasri, M., (2006) study results show that with increasing drought stress at different growth stages, amount of RWC is reduced (Table 1). Leaf RWC was significantly decreased by drought stress. Based on mean comparison highest RWC was found in Kranti and lowest in Pusa Bold. Because, relative water content contains amount of available water in leaf, increasing stress causes to decreasing it. However, greater reduction was observed in drought sensitive varieties (Ullah *et al.,* 2012) which are in agreement with the results of this study.

We observed that electrolyte leakage found to be dependent on severity of drought stress. Genotype Pusa Bold shows maximum electrolyte leakage and minimum was shown in Kranti (Table 1). These results agreed with the findings ofAli *et al.,* (2013) and Quan *et al.*, (2004). Among the cultivars, Kranti had the lowest values for theses parameters. In mungbean Kumar *et al.*, (2013) reported that cell membrane stability has been widely used to express stress tolerance in plants and higher membrane stability is correlated with stress tolerance by Premchandran *et al.,* (1990).

There isdecline in pollen viability after exposure to drought stress at different DAS in all genotypes was observed; percent decline in viability was highest in Pusa Bold followed by NRCDR-2, RSPR-01, RSPR-03 and lower percent was observed in Kranti (Fig.1). These results confirmed the earlier findings of (Canci and Toker, 2009; Krishnamurthy *et al*., 2011; Upadhyaya *et al*., 2011). Similar results were also observed in *Phaseolus vulgaris* (Porch and Jahn, 2001). The reduction in pollen viability might be related to their developmental impairment (Porch and Jahn, 2001) or lack of sufficient starch at maturity (Pressman *et al*., 2006).

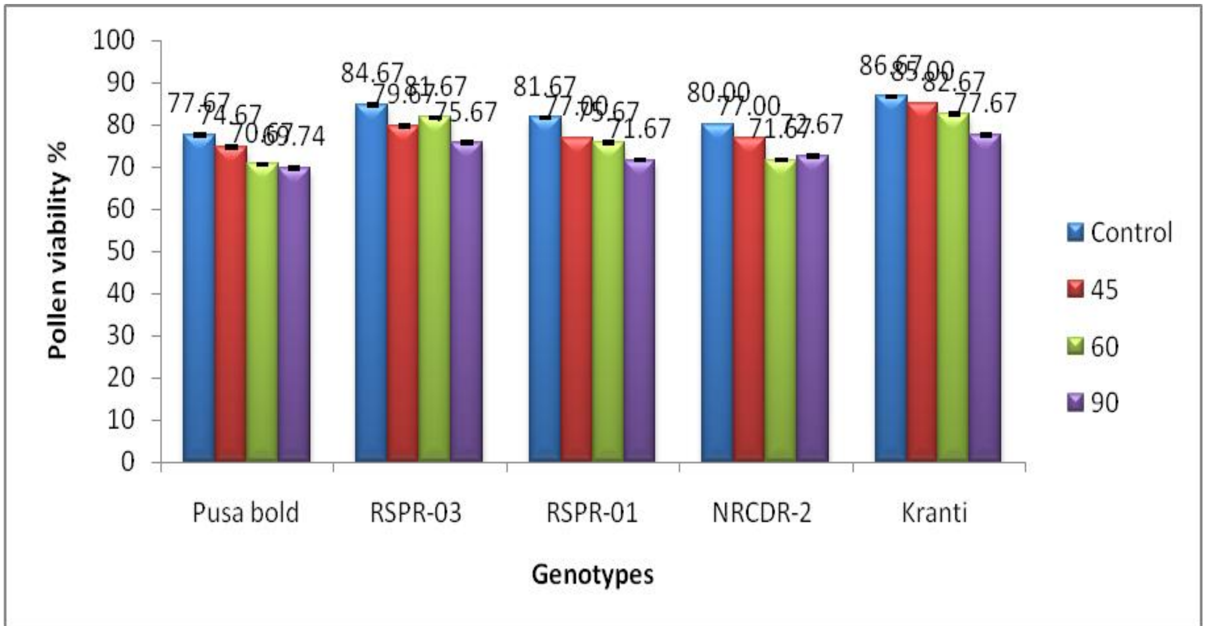
In this result Kranti retained more number of flowers and pods under drought stress followed by RSPR-03 and minimum was in Pusa Bold. These findings confirm with results of Upadhyaya *et al*., (2011). Wang *et al*., (2006) also noted that chickpea plant biomass and number of seeds per plantwere reduced under high temperature. Similar results were observed in ground nut (Prasad *et al*., 2000; 2001) and common bean (Gross and Kigel, 1994).

The seed yield per plant of *B. juncea* is a cumulative effect of various yield components like number of podsper plant, number of seeds per pods, and grain yield per plant. The data regarding seed yield of different *B. juncea* cultivars given in the Table 1 revealed that grain yield and its components were significantly affected by drought stress applied at flower initiation and pods filling stages.

Table.1 Changes in physiological, phenological and reproductive behaviour of *Brassica juncea* genotypes as affected by drought stress

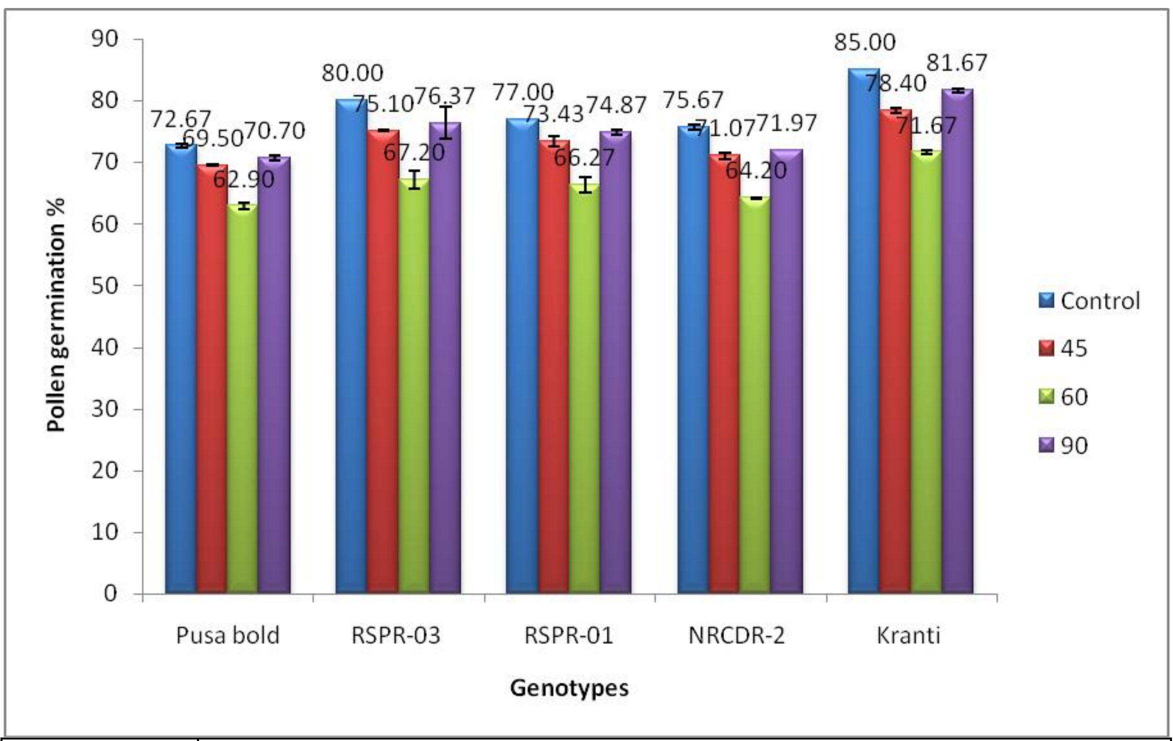
Treatments	Genotypes	Relative Water Content (RWC)%	Relative Stress Injury (RSI)%	Flower shedding (%)	Days to 50 % Flowering	Days to 50 % Pod formation	Days to physiological maturity	Yield plant^{-1} (g)
Control	**Pusa bold**	70.43±1.35	24.31±0.98	28.32±0.16	76.6± 0.58	94.8±0.29	126.1±0.23	3.30±0.18
	RSPR-03	75.10±1.04	20.30±0.96	20.76±0.14	70.6± 0.46	89.1±0.12	120.1±0.29	4.78±0.18
	RSPR-01	75.03±0.74	22.46±0.07	23.02±0.03	73.1± 0.29	91.1±0.35	122.2±0.12	4.34±0.17
	NRCDR-2	73.79±0.25	23.92±0.16	25.99±0.40	74.0± 0.23	93.1±0.29	124.1±0.52	4.22±0.04
	Kranti	79.20±0.01	19.95±1.15	18.48±0.16	69.0± 0.58	87.1±0.23	119.1±0.17	4.93±0.32
45 DAS	**Pusa bold**	43.47±0.68	32.30±1.12	30.34±0.14	74.7± 0.29	91.1±0.35	122.2±0.17	2.53±0.44
	RSPR-03	57.00±0.68	28.45±0.34	28.26±0.03	69.1± 0.00	85.2±0.12	116.1±0.17	3.57±0.05
	RSPR-01	54.04±0.93	31.68±0.86	27.86±0.40	71.0± 0.06	87.2±0.06	118.1±0.29	3.41±0.26
	NRCDR-2	43.67±0.06	31.89±0.82	30.56±0.16	73.0± 0.29	90.1±0.12	121.0±0.35	2.73±0.31
	Kranti	57.44±0.63	24.52±0.17	23.68±0.14	65.1± 0.00	84.3±0.29	115.1±0.12	4.59±0.05
60 DAS	**Pusa bold**	42.93±0.57	40.18±0.07	38.24±0.03	71.8± 0.12	89.2±0.06	120.1±0.06	2.22±0.08
	RSPR-03	51.06±2.19	29.25±0.26	35.60±0.40	67.0± 0.06	83.1±0.35	114.1±0.17	3.40±0.06
	RSPR-01	50.73±1.61	32.12±3.37	37.50±0.16	69.0± 0.00	85.0±0.23	116.0±0.17	3.36±0.12
	NRCDR-2	43.06±0.16	32.44±0.24	36.25±0.14	71.0± 0.12	87.2±0.23	118.0±0.06	2.70±0.06
	Kranti	53.93±0.91	25.77±0.11	32.32±0.03	64.1± 0.00	82.0±0.29	113.0±0.00	4.45±0.02
90 DAS	**Pusa bold**	33.90±0.21	47.32±0.25	29.73±0.40	76.1± 0.06	93.2±0.23	123.7±0.35	2.91±0.16
	RSPR-03	40.08±0.50	35.19±0.67	24.72±0.16	70.0± 0.00	87.0±0.12	118.2±0.12	4.27±0.04
	RSPR-01	38.28±0.32	42.20±0.03	27.72±0.14	72.0± 0.29	89.2±0.50	120.1±0.17	3.68±0.15
	NRCDR-2	33.96±0.47	45.41±0.35	27.23±0.03	72.0± 0.29	91.1±0.00	122.2±0.06	3.29±0.09
	Kranti	47.44±0.39	32.13±0.78	21.08±0.40	68.0± 0.00	85.0±0.06	116.0±0.00	4.66±0.14
C.D. at 5%	*Genotypes*	*0.717*	*0.803*	*0.084*	*0.218*	*0.204*	*0.182*	*0.667*
	Drought	*0.641*	*0.718*	*0.076*	*0.195*	*0.183*	*0.162*	*0.597*
	Genotypes X Drought	*1.434*	*1.606*	*0.169*	*0.436*	*0.409*	*0.363*	*N/A*

Mean ± standard deviation



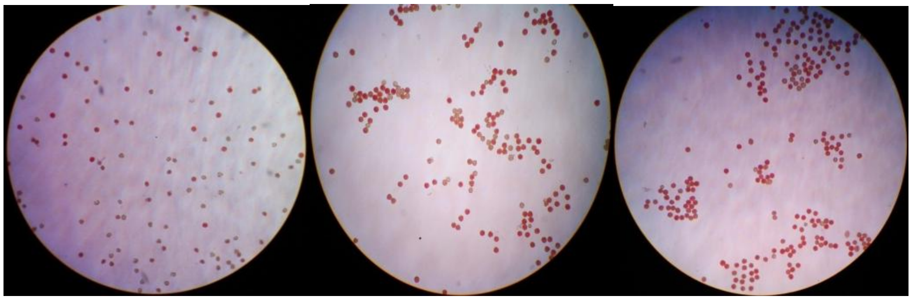
C.D. at 5%	*Genotypes* =*0.42* *Drought* =*0.38* *Genotypes X Drought* =*0.84*

Fig.1 Changes in pollen viability of *B. juncea* genotypes after exposure to drought stress

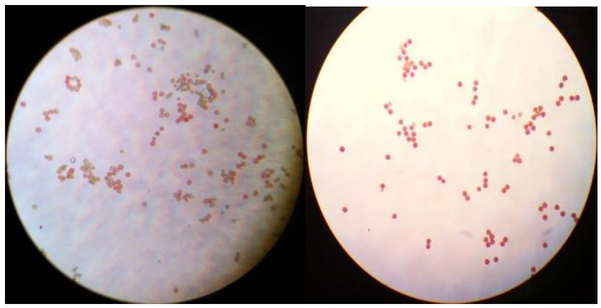


C.D. at 5%	*Genotypes* =*1.12* *Drought* =*1.00* *Genotypes X Drought* =*NS*

Fig.2 Changes in pollen germination% of *B. juncea* genotypes after exposure to drought stress



Pusa BoldRSPR-03Kranti



NRCDR-2 RSPR-01

Figure.3 Pollen viability of *Brassica juncea* genotypes

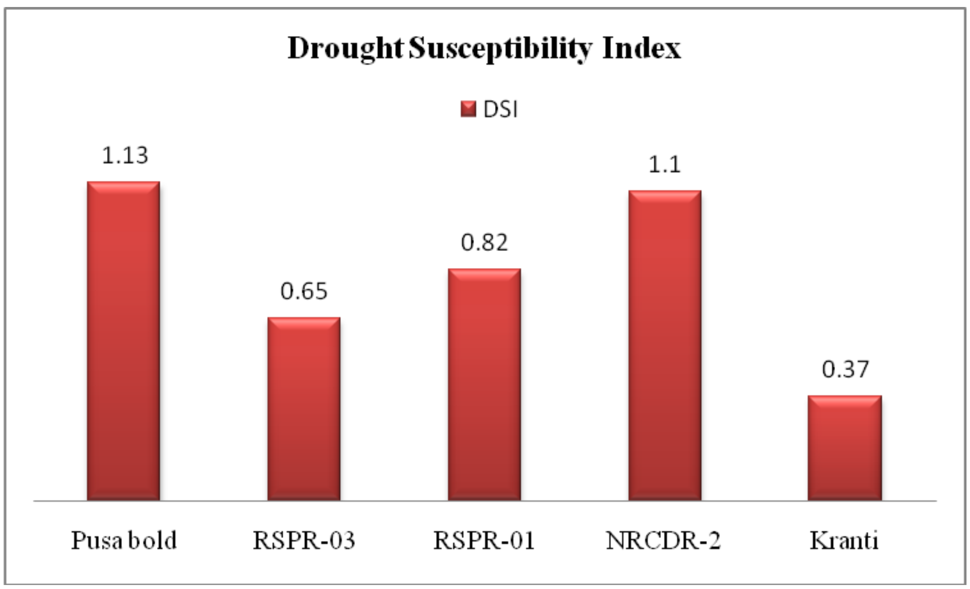


Fig.4 Drought susceptibility index*B. juncea* genotypes under drought stress condition

The reduction was greater when drought stress was applied at flowering stage. Water stress during flowering and early pod development reduces yield of *Brassica napus* and *Brassica campestris* by reducing pods number and seed per pod. Similar reduction in seed yield was also observed by Maharaj *et al.*, (2003) in mustard genotypes. Richards and Thurling, (1978) also showed that the drought markedly influenced seed yield in spring cultivars of oilseed rape species (*Brassica compestris* and *Brassica napus* L.).

Days to maturity decreased with delay in sowing. These findings are similar to those of Yadav *et al*., (1995) who reported that days to maturity decreased with delay in sowing time. Krishnamurthy *et al*., (2011) suggested that days to 50% flowering was delayed and days to maturity was hastened at high temperature, but Upadhyaya *et al*., (2011) observed early flowering and forced maturity. However, the present study and previous studies observed a shortened period of grain filling due to accelerated rate of plant development (Gan *et al*., 2004). Therefore the grain yield under heat stress was reduced due to lack of assimilate partitioning from leaves to seed (Wardlaw, 1976).

This finding was consistent with the reports of Zakirullah *et al*., (2000) and Sinaki *et al*., (2007). In general, the reaction of crops and their evaluation for an optimum yield under different environmental conditions depend on their ability to use the said conditions. This would be possible through regulating yield components and the interaction of genotype with the environment when desirable and undesirable conditions occur in each stage of plant growth and development (Entz, Flower, 1990).

Based on physiological traits (Relative water content), and phenological and reproductive behavior such as pollen viability and seed yield, it is concluded that Kranti, followed by RSPR-03 and RSPR-01 showed better performance under drought stress as compared to Pusa Bold and NRCDR-2. These results suggested that selection for above studied characters could improve the drought tolerance of *Brassica juncea*. These characters can be useful selection criteria in breeding programme for increasing the grain yield of *Brassica juncea*.

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