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Physiological and biochemical traits as tools to screen sensitive and resistant genotypes of *Brassica juncea* exposed to drought stress

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Abstract

Impact of drought stress on physiological and biochemical changes were evaluated in five *Brassica juncea* genotypes at three different growth stages in a pot experiment i.e., water stress imposed at branch initiation, flower initiation and siliquae formation stages in the form of 45, 60 and 90 (DAS) days after sowing. The study revealed that heat stress decreased chlorophyll content, chlorophyll stability index (CSI) and relative water content (RWC) in all the genotypes. In contrast, drought stress induced an increase in proline, relative stress injury (RSI) and soluble sugar content in all genotypes. The drought susceptibility index (DSI) for seed yield and its attributes were recorded to characterize the relative tolerance of genotypes which clearly distinguish *Brassica juncea* genotypes into drought tolerant and susceptible cultivars. Noteworthy in comparison to the drought-sensitive varieties, *Brassica* genotype that possessed higher degrees of drought tolerance Genotypes Kranti displayed higher chlorophyll content, CSI, RWC, soluble sugar content, better osmoregulating ability with the increase in proline content and lower level of membrane injury index. The findings of the present research investigation recommended the growing of Kranti genotypes in the drought prone areas to obtain high economic yield even in adverse condition.

Keywords: *Brassica juncea*, chlorophyll content, RWC, soluble sugars, proline

Introduction

Rapeseed-mustard is generally grown under rain fed condition in Jammu region and drought plays vital role in determining the yield of the crop. Drought is one of the environmental factors, which influence the growth in every phenophase of the crop cycle. Its impact on morphology, development, biomass production and days to attain the phenophase is well established. The full genetic potential of the cultivar is only obtained when an optimum climatic condition including other environmental factors is available. The drought conditions during early and later stages caused by higher temperatures coupled with moisture stress adversely affect growth and yield of mustard crop.

Drought severely affects plant growth and development with substantial reductions in crop growth rate and biomass accumulation. Relative water content is the ability of plant to maintain high water in the leaves under moisture stress conditions and has been used as an index to determine drought (Basker 2013; Gurdev 2015) [5, 20] tolerance in crop plants. Relative water content is considered a measure of plant water status, it is essential because it expresses the absolute amount of water which the plant requires to reach full artificial saturation and reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. 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.* 2003; Karim *et al.* 2003) [9, 24].

The effect of drought stress on chlorophyll depends on plant species and stress conditions. Reduction of chlorophyll content has been considered as a commonly observed phenomenon in response to drought stress (Bayat *et al.* 2009) [6]. Drought stress not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes, therefore reduction in chlorophyll contents is expected (Ladjal *et al.* 2000) [29]. In order to deal with this effect, plants have evolved a number of protective defence mechanisms. Various metabolites accumulate in plant tissues upon reduction in leaf water status. Researchers reported that proline is one of the compatible solutes that accumulate in response to water stress and the accumulation of these osmolytes represents an important adaptive response to salt and water stress. Proline act as an osmo-regulator, maintain membrane integrity and affect the solubility of various proteins due to its interaction with hydrophobic residues on the protein surface under the conditions of reduced water availability (Farooq *et al.* 2008; Kohila *et al.* 2017) [13, 25].

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) [16]. 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. 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; Greaves and Wang 2017) [15, 46, 17]. All these factors contribute to reduced dry matter accumulation and grain yield under drought.

Therefore, the present research was conducted to study the behaviour of *Brassica juncea* under drought stress conditions, with reference to the physiological, biochemical changes like relative water content (RWC%), relative stress injury, proline accumulation, chlorophyll content and chlorophyll stability index in *brassica* is very important for identifying drought-tolerant traits that can be incorporated into high-yielding varieties. These findings could then be used to predict the susceptibility or tolerance of a cultivar as well as suitability of planting under drought stress conditions. The aim of this study was to determine the effect of drought stress at different growth stages on qualitative and quantities traits of five *Brassica juncea* genotypes.

Materials and methods

Plant materials and growth conditions (Treatments)

Five *Brassica juncea* 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. 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. Sowing was done in the last week of October. Seedlings were thinned to 3 per pot at three leaf stage. 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) [43, 45]. The control plants were irrigated continuously at the optimum moisture regime. Five plants from each plot were randomly chosen for recording observations on their physiological, biochemical and yield attributing characters. The effect of drought was assessed as percentage reduction in mean performance of a characteristic under rain-fed conditions relative to the performance of the same trait under irrigated conditions.

Methodology

Quantitative analysis of pigment content

Chlorophyll was extracted by the non-destructive method described by Gunes *et al.*, (2007) [18] using dimethyl sulphoxide (DMSO) and absorbance was read at 665, 645 and 454 nm as described by Kaloyereas (1958) [23] on a computer aided spectrophotometer (Systronic India Spectrophotometer 117) running a multiple wave length programme. DMSO was used as blank. Calculations for different pigments were made according to the formulae (Lichtenthaler 1987) [30] given as: Chlorophyll *a* (mg/g FW) = (11.75 X A_{665} - 2.35 X A_{645}) X 3/30, Chlorophyll *b* (mg/g FW) = (18.61 X A_{645} - 3.96 X A_{665}) X 3/30. Quantities of these pigments were calculated in mg g^{-1} tissue fresh weight. Total chlorophyll = Chl *a* + Chl *b*. Chlorophyll Stability Index (CSI) was estimated by Kaloyereas (1958) [23] and the chlorophyll content variations between the control and treatment were calculated as CSI.CSI = Total chlorophyll of heated samples / Total chlorophyll of non- heated samples. SPAD values of leaves were recorded as described by Peng *et al.*, (1993) [37] using the chlorophyll meter (SPAD - 502, Soil Plant analysis Development Section, Minolta Camera Co. Ltd., Japan).The RWC was calculated using the formula (Weatherley 1950). RWC % = [(fresh weight - dry weight)/ (turgid weight - dry weight)] x 100.

Biochemical analysis

Proline content was estimated by using the method of Bates *et al.*, (1973) [4]. Standard curve was prepared by using graded concentration of proline in 3% sulphosalicylic acid. The proline content was expressed as μ mol g^{-1} FW. Total soluble carbohydrates were determined with the method of Yemm and Willis (1954) [49] using anthrone reagent. Standard curve was prepared using graded concentration of D-glucose and the data were expressed as g FW g-1. The equation of the standard curve was y = 1.222x - 0.001, R^2 = 0.9944. The relative stress injury (RSI %) in leaves was evaluated by (Sullivan 1972). The relative stress injury (RSI) was calculated as follows: RSI (%) = 1- EC_a / EC_b x 100.

Statistical analysis

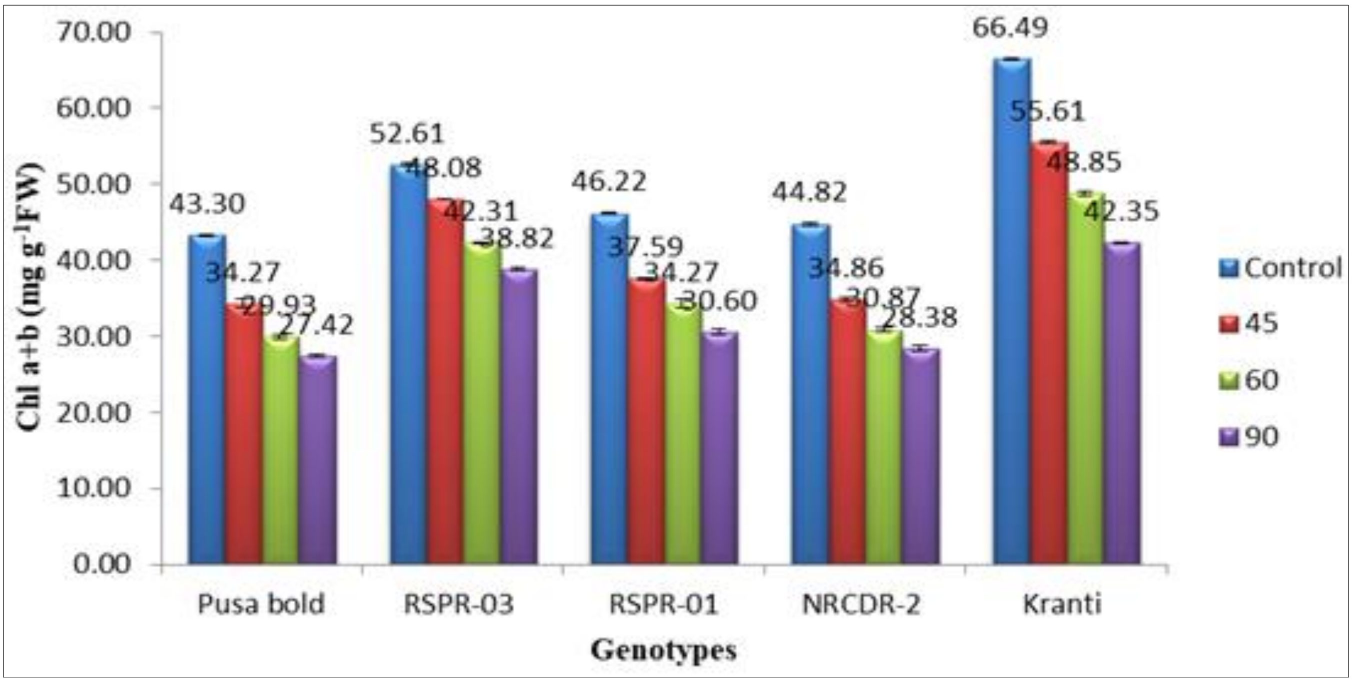
For each parameter, three pots having three plants per pot were sampled at a time which comprises of three replicates. Data were analysed using Completely Randomized Block Design (CRBD) for two factors. Treatments were compared using critical difference (CD) at 5 % level of significance. Data were subjected to analysis of variance (ANOVA) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar 125 004, Haryana, India).

Result and Discussion

The growth and yield of rapeseed-mustard is influenced by various agro-eco conditions to a greater extant from which the influence of drought is more prominently viewed and even higher temperature by 3-4^{o}C during pod formation and seed filling stage affect yield of mustard considerably. The drought conditions during early and later stages caused by higher temperatures coupled with moisture stress adversely affect growth of mustard crop. In the present study, biochemical characterization of five *Brassica juncea* genotypes was undertaken for differences in their response to drought stresses. Mustard crop in the field is frequently subjected to drought stresses that affect adversely their growth, development and productivity.

Chlorophyll content (mg g-1FW)

Drought stress reduced the total chlorophyll contents in all *B. juncea* genotypes in all three growth stages *i.e.*, 45, 60 and 90 DAS (Fig. 1). Reduction of chlorophyll content has been considered as a commonly observed phenomenon in response to drought stress (Bayat *et al.* 2009) [6]. Drought not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes, therefore reduction in chlorophyll contents is expected (Ladjal *et al.* 2000) [29]. Decrease in chlorophyll content under water stress is a commonly observed phenomenon (Chaves *et al.* 2003; Reynolds *et al.* 2005; Gurdev 2015; Dinh *et al.* 2017) [9, 41, 20, 12]. Plants under water stress condition accumulate less chlorophyll content than the unstressed plants. Chlorophyll content in *B. juncea* genotypes showed varied response to water stress. In *B. juncea* genotypes Kranti and RSPR-03 showed less reduction in chlorophyll content under stressed conditions than other genotypes. Values of chlorophyll content declined at siliquae formation stage as chlorophyll content in leaves depends upon the age of the plant. Singh *et al.*, (2003) [47] studied mustard genotypes under irrigated and rain fed conditions and found that chlorophyll content decreased during all the growth stages under rain fed. The variation in chlorophyll between the cultivars might be due to specific chlorophyll synthesizing enzymes such as chlorophyllase (Majumdar *et al.* 1991) [32]. Singh *et al.*, (2003) [47] found a significant reduction in chlorophyll contents in the leaves of mustard genotypes under drought stress. Paclik *et al.*, (1996) [35] observed that water stress reduced chlorophyll a and b contents by 38% compared with the adequately watered plants.

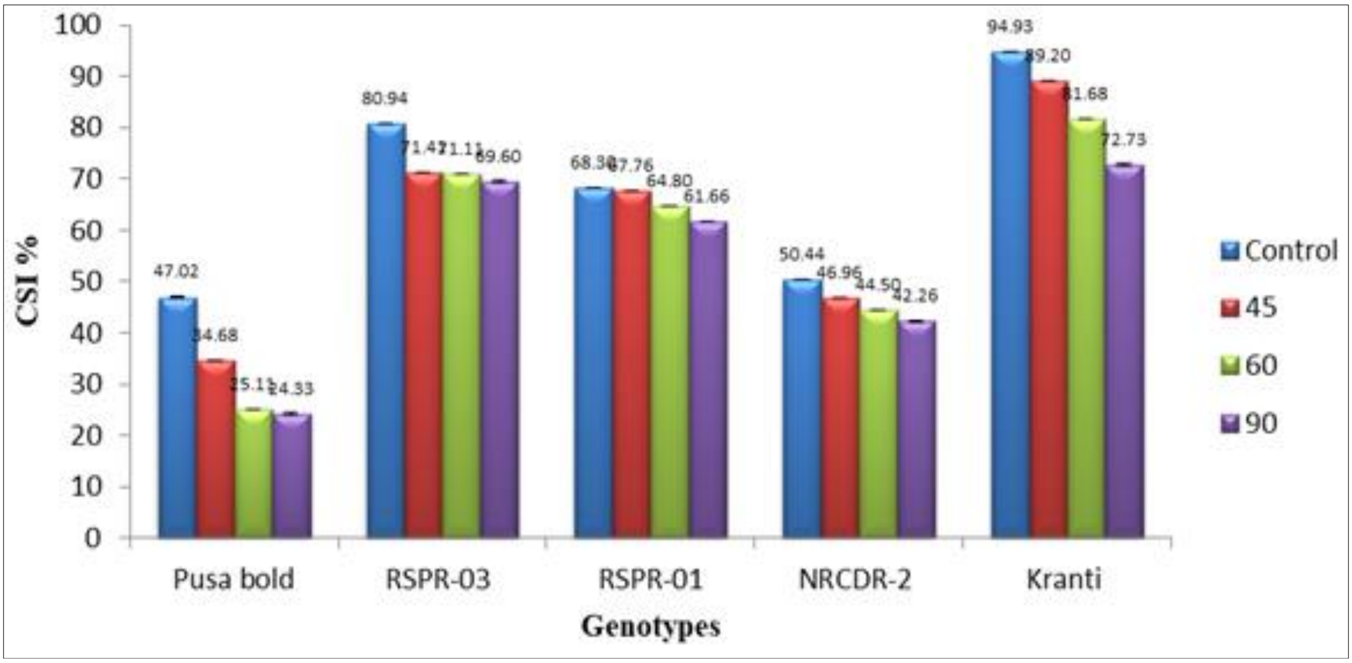


C.D. at 5%	Genotypes	=0.411
	Drought	=0.368
	Genotypes X Drought	=0.823

Fig 1: Changes in chlorophyll (*a+b*) content of leaves in *B. juncea* genotypes as exposed to drought stress.

Chlorophyll stability index (%)

The data of chlorophyll stability index (CSI %) revealed that genotypes with maximum chlorophyll stability index showed drought tolerance as compared to unstressed plants. Chlorophyll stability index (CSI %) (Fig. 2) decreased with drought stress at different DAS in all five genotypes and the values varied from 68.32 % to 54.11%. Genotypes Kranti and RSPR-03 showed maximum chlorophyll stability index than genotypes RSPR-01, NRCDR-2 and Pusa Bold under stressed condition. The results were in agreement with Gadallah (1995) [14] who studied the effect of dehydration stress on chlorophyll content, chlorophyll stability index, leaf relative water content in cotton plant (*Gossypium barbadens*) and reported that the membrane of water stressed plants was less stable than that of unstressed plants. The results of this experiment indicated that stress at flowering stage is more sensitive to water deficit due to number of pods per plant and number of seeds per pod. Selection for yield in these *Brassica juncea* genotypes growing under drought stressed conditions is therefore predicted to be more efficient if performed in a stressed environment. However, selection criteria alternative to yield may prove more useful in yield improvement programs than yield itself.



C.D. at 5%	Genotypes	=0.167
	Drought	=0.150
	Genotypes X Drought	= 0.335

Fig 2: Changes in chlorophyll stability index (CSI %) of leaves in *B. juncea* genotypes as affected by drought stress.

Relative water content (%)

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) [44]. The relative water content decrease significantly to drought stress compared with control. Our observations are in agreement with other researchers. Loon C.D. (1981) [31] study results show that with increasing drought stress at different growth stages, amount of RWC is reduced. Leaf RWC was significantly decreased by drought stress. Our results (Table1) showed that, in all five genotypes relative water content was affected at different DAS. Lowest average of RWC observed in 90 DAS. 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. Different rape seed varieties were grew under various irrigation regimes and reduction in value of RWC of rape seed was recorded with depleting irrigation. RWC was decreased in both drought tolerant and sensitive varieties. However, greater reduction was observed in drought sensitive varieties (Ullah *et al.* 2012) [48] which were in agreement with the results of this study.

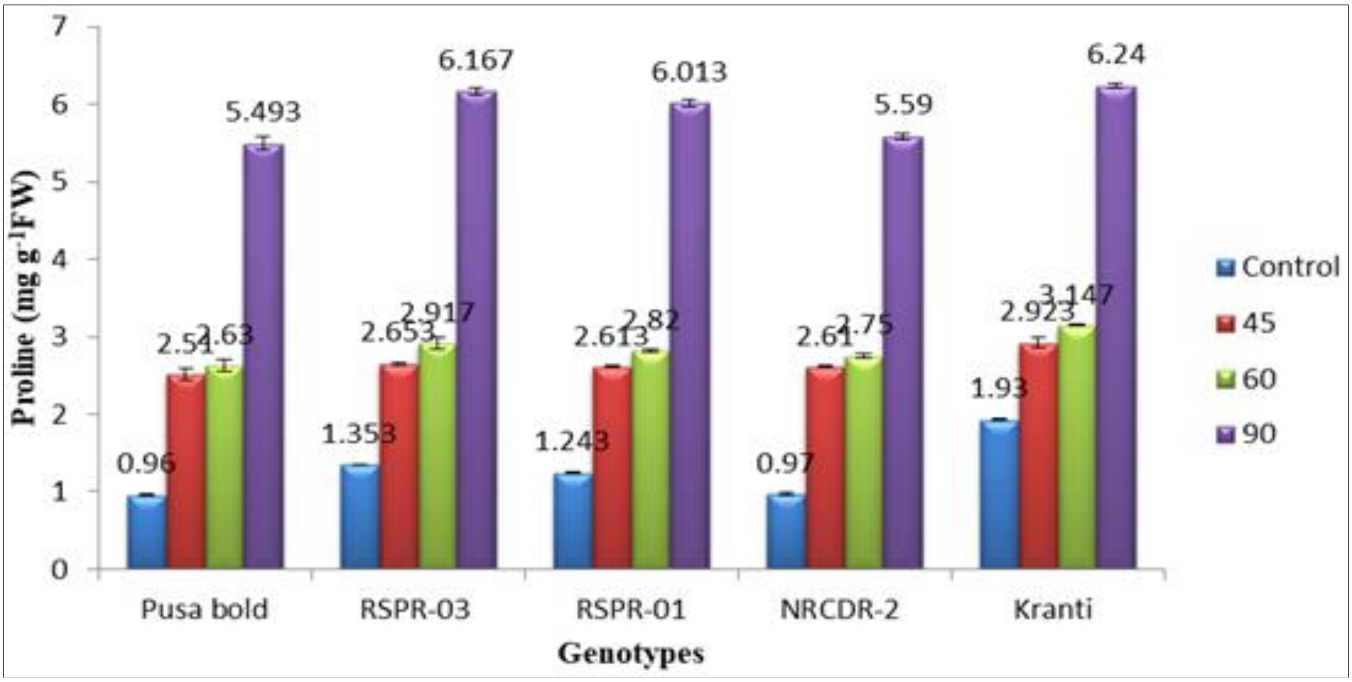
Table 1: Changes in relative water content of leaves in *B. juncea* genotypes as exposed by drought stress (RWC %)

Relative Water Content (RWC)%					
Genotypes	Control	Drought stress (DAS)			Mean
		45	60	90	
Pusa bold	70.43	43.47	42.93	33.90	47.68
RSPR-03	75.10	57.00	51.06	40.08	55.81
RSPR-01	75.03	54.04	50.73	38.28	54.52
NRCDR-2	73.79	43.67	43.06	33.96	48.62
Kranti	79.20	57.44	53.93	47.44	59.50
Mean	74.71	51.12	48.34	38.73	
C.D. at 5%	Genotypes			=0.717	
	Drought			=0.641	
	Genotypes X Drought			= 1.434	

Biochemical parameters

Proline content (mg g-1FW)

It has been well known, that metabolites like proline, sugars accumulate to a high level in plants when they are under stresses such as drought, high temperature, salinity and other environmental stresses (Ashraf and Foolad 2007; Kovacik *et al.* 2009; Parween *et al.* 2012; Afzal *et al.* 2014) [1, 3, 26, 36]. In the present investigation, proline content increased markedly in leaves in all five *B. juncea* genotypes (Fig. 3). The data showed significant differences in proline content of leaves and it increased from control to 90 DAS of drought stress *i.e.*, 1.29 to 5.90 respectively. The highest proline content was observed in Kranti followed by RSPR-03 and RSPR-01 and lowest in NRCDR-2 and Pusa Bold at 90 DAS as compared to control and under drought stress condition. Same result was observed in non-stressed condition (control) in different growth stages, but proline was higher in stressed plants and proline content in leaf decreased significantly after irrigation. Increase in proline during stress in *Brassica* is in agreement with Hsu *et al.*, (2003) [21]; Gunes *et al.*, (2008) [19]; Din *et al.*, (2011) [11].

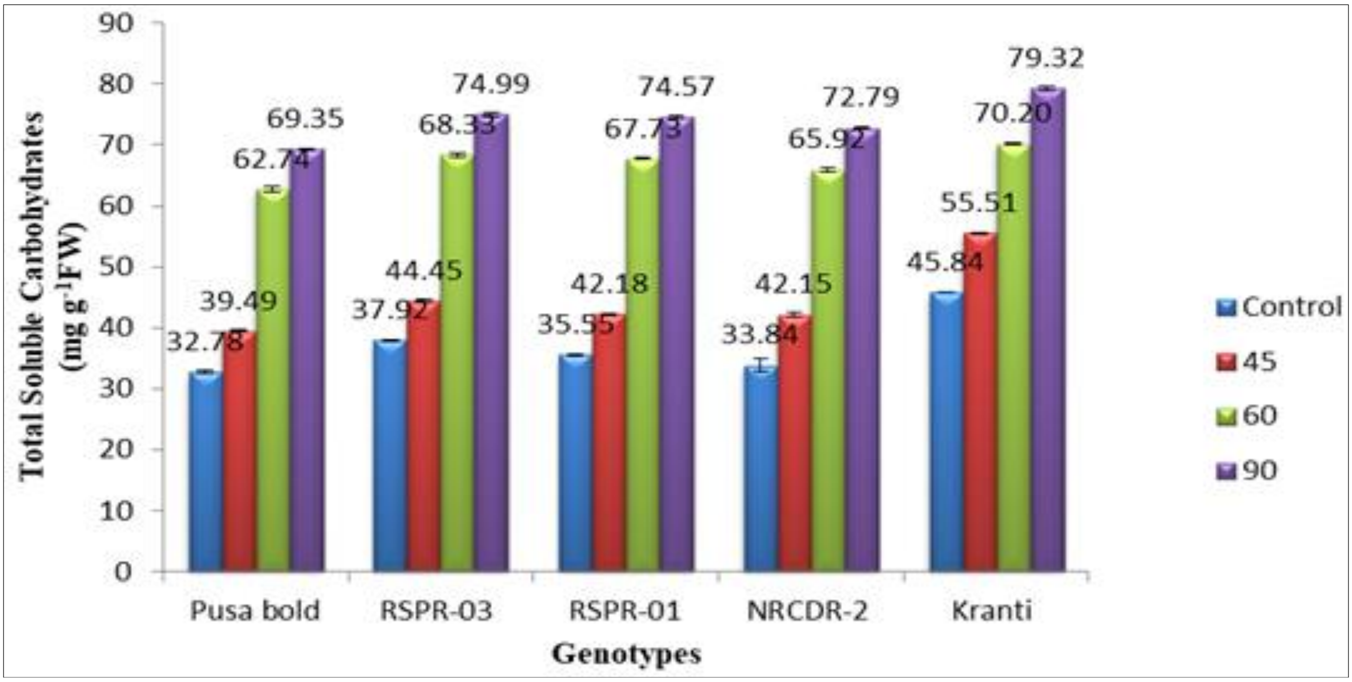


C.D. at 5%	Genotypes	=0.062
	Drought	=0.056
	Genotypes X Drought	= 0.124

Fig 3: Proline content in *B. juncea* leaves after exposure to drought stress conditions.

Total soluble sugars (mg g^{-1}FW)

The influence of drought increases the total soluble carbohydrates TSC in leaves of *Brassica* genotypes (Fig. 4). Similar to proline, the overall accumulation of TSC was more in leaves of Kranti at 90 DAS to drought stress. The TSC increased significantly with every increment of exposed day to drought stress. The genotype Kranti maintained higher TSC followed by RSPR-03 and minimum in NRCDR-2 and Pusa Bold. The amount of TSC increased rapidly to the increasing high temperature, this result agree with the result of some researchers who indicated that drought (Jebory 2012 [22], Naresh *et al.* 2013) [34] and salinity stress induced soluble carbohydrates accumulation in chickpea (Nandwal *et al.* 2007; Kukreja *et al.* 2010; Chand *et al.* 2015; Gurdev 2015) [20, 33, 27, 7]. As our results and earlier reports on increasing in accumulation of soluble carbohydrates, we concluded that these osmotic compatible solutes increase the heat tolerance of *Brassica* plants by maintaining the higher RWC in leaves and stabilization of essential protective enzymes proteins, resulting in better physiological activity.



C.D. at 5%	Genotypes	=0.51
	Drought	=0.45
	Genotypes X Drought	= 1.01

Fig 4: Total soluble carbohydrates (TSC) in *B. juncea* leaves after exposure to drought stress.

Relative stress injury (RSI %)

Leakage of electrolytes (Table 2) increased significantly with increased days of exposure at different growth phases to drought stress in all *B. juncea* genotypes. 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. These results agreed with the findings of Quan *et al.*, (2004) [39], Ali *et al.*, (2013) [2] and Devi *et al.*, (2013) [10]. Among the cultivars, Kranti had the lowest values for theses parameters. In mungbean Kumar *et al.*, (2013) [28] 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 Premachandra *et al.*, (1990) [38].

Table 2: Changes in relative stress injury of leaves in *B. juncea* genotypes as exposed to drought stress

Relative Stress Injury (RSI)%					
Genotypes	**Control**	**Drought stress (DAS)**			Mean
		45	**60**	**90**	
Pusa bold	24.31	32.30	40.18	47.32	36.02
RSPR-03	20.30	28.45	29.25	35.19	28.30
RSPR-01	22.46	31.68	32.12	42.20	32.11
NRCDR-2	23.92	31.89	32.44	45.41	33.41
Kranti	19.95	24.52	25.77	32.13	25.59
Mean	22.19	29.77	31.95	40.45	
C.D. at 5%	Genotypes			=0.803	
	Drought			=0.718	
	Genotypes x Drought			= 1.606	

Drought susceptibility index

The lowest drought susceptibility value was shown by the drought tolerant genotype while highest drought susceptibility index was recorded in drought susceptible genotype. Drought susceptibility index showed decreasing trend in all the genotypes from branch initiation stage to siliquae formation stage (Fig. 5). Genotype Kranti showed significantly less value of DSI. Sadaqat *et al.*, (2003) [42] and Chauhan *et al.*, (2007) [8] reported that drought tolerant varieties showed minimum drought susceptibility index.

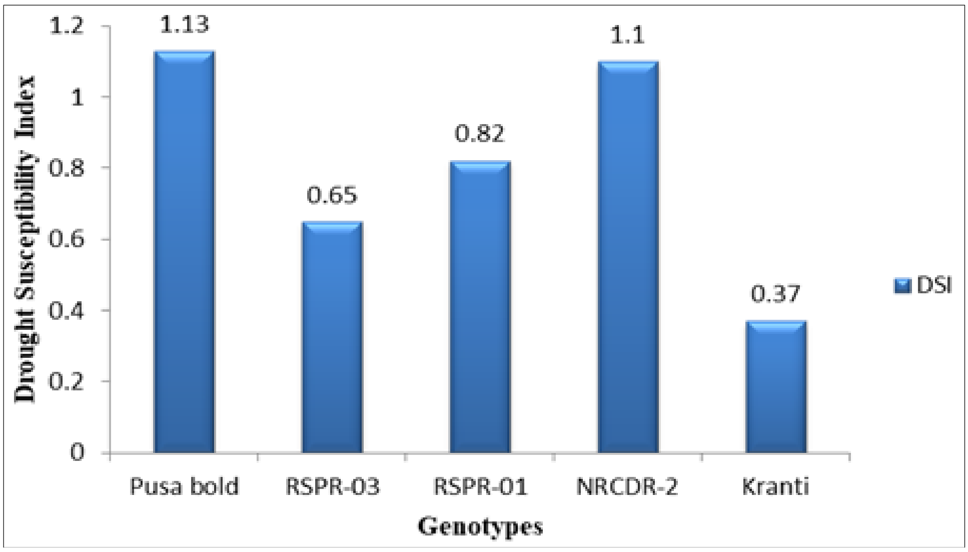


Fig 5: Drought susceptibility index (DSI) of *B. juncea* genotypes under drought stress condition

Conclusion

In conclusion, the growth and yield of rapeseed-mustard are influenced by various agro-eco conditions to a greater extant from which the influence of drought is more prominently viewed during pod formation and seed filling stage affect yield of mustard considerably. The drought conditions during early and later stages caused by drought stress adversely affect growth of mustard crop. This study showed that physiological parameters such as chlorophyll content, CSI, soluble sugar content and proline content could be used as supplementary or alternative indicators for drought tolerance in *brassica juncea*. Among the genotypes studied, it was concluded that genotypes Kranti and RSPR-03 proved to be tolerant to drought stress, which can be used as donor genotype for drought tolerance. Genotypes RSPR-01 and NRCDR-2 were moderately drought tolerant, whereas genotypes Pusa Bold was susceptible to drought stress.

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