

## EFFECTS OF PLANT GROWTH REGULATORS ON GROWTH AND OIL QUALITY OF CANOLA (*BRASSICA NAPUS* L.) UNDER DROUGHT STRESS

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### Abstract

Growth regulators have previously been documented to enhance growth and improve oil and protein content of plants. This paper documents the effects of Salicylic acid (SA) and Putrescine (Put) on growth and oil quality of canola (*Brassica napus* L.) when exposed to drought stress. Two canola cultivars cvv. Rainbow and Dunkeld were grown under natural environmental conditions. Drought stress was imposed for 10d during flowering (90 days after sowing) until the soil moisture content decreased from 22%-9%. The growth regulators salicylic acid and Putrescine were applied @  $10^{-5}$  mol/L as foliar spray 3 days after drought induction. Drought stress significantly reduced leaf relative water content (LRWC), chl *a*, chl *b*, carotenoids and soluble proteins but augmented the leaf proline, seed glucosinolates and oil erucic acid (C22:1) contents. Growth regulators were highly effective in ameliorating the adverse effects of drought stress on both the canola cultivars. The applied growth regulators maintained the water budget of canola plants, augmented the accumulation of osmolyte proline and protected photosynthetic pigments from adverse effects of drought stress. The SA was effective to reduce the drought induced accumulation of glucosinolates and erucic acid in canola oil and both the growth regulators overcame the drought induced decrease in oleic acid (C18:1). It is inferred that SA is economical and environment friendly alternative and can be implicated to improve the plant growth and oil quality of canola in current scenario of drought and climate change.

### Introduction

Among the different abiotic stresses like heat, salinity and freezing, drought stress is a more severe constraint that limits growth and productivity of crop plants (Yamaguchi-Shinozaki *et al.*, 2002). Drought is a world-spread problem seriously reducing the yield and quality of crop plants (Hongbo *et al.*, 2005). It affects every aspect of plant physiology, biochemistry and diminishes yields (Moghadam *et al.*, 2011).

Canola (*Brassica napus* L.) is considered as an economically important crop of Pakistan. But erratic rainfall and scarcity of water for irrigation during the growing season significantly lowers its yield and quality. Water stress affects both vegetative and reproductive stages in canola. The effects of water stress were more severe during reproductive growth than vegetative growth in rapeseed (Ghobadi *et al.*, 2006). Previous studies showed that drought stress significantly decreased the seed oil content of canola (Sinaki *et al.*, 2007). Similarly, Pham-Thi *et al.*, (1985) reported that water deficiency decreased the degree of fatty acids unsaturation which was attributed to the inhibition in the biosynthesis of polyunsaturated fatty acids and suppression in the activities of desaturases.

Plant growth regulators like salicylic acid, cytokinin, gibberellins and abscisic acid amend the plant responses towards drought stress (Farooq *et al.*, 2009). Salicylic acid plays diverse physiological roles in plants which includes plant growth, thermogenesis, flower induction, nutrient uptake, ethylene biosynthesis, stomatal movements, photosynthesis and enzyme activities (Hayat & Ahmed, 2007). Foliar application of salicylic acid ameliorates the adverse effects of water stress and enhances the restoration of the growth process in wheat (Sakhabutdinova *et al.*, 2003). Putrescine, a new plant growth regulator, belonging to polyamines, is reported for its beneficial effects on plant growth under environmental stresses (Perez-Adamor *et al.*, 2002).

Salicylic acid and Putrescine are economical, non-hazardous and bring stress resistance through metabolic defense mechanisms leading to better plant growth and yields. Therefore, the aim of the present investigation was to determine the possible role of plant growth regulators on growth and oil quality of canola under drought stress.

### Materials and methods

**Plant material and growing conditions:** Seeds of two canola cultivars viz cvv. Rainbow and Dunkeld obtained from National Agriculture Research Center, Islamabad, were surface sterilized with 10% chlorox solution and grown in earthen pots (27x30cm<sup>2</sup>) filled with a mixture of clay, sand and farmyard manure (6:1:1) under natural environmental conditions during canola growing season of 2009-2010. Drought stress was imposed during flowering for 10 days by withholding water supply until the soil moisture was reduced from 22-9%. The growth regulators (salicylic acid and putrescine) were applied at  $10^{-5}$  mol/L as foliar spray 3d after imposition of drought stress. The experiment was laid out in complete randomized design (CRD) with three replica of each treatment. Several treatments were made to evaluate the effects of growth regulators under drought stress.

C-Control, T1-Drought, T3-Salicylic acid ( $10^{-5}$  mol/L), T4-Putrescine ( $10^{-5}$  mol/L), T5-Drought + Salicylic acid ( $10^{-5}$  mol/L), T6-Drought + Putrescine ( $10^{-5}$  mol/L).

Soil moisture (%) was determined before and after induction of drought.

$$\% \text{ Soil moisture} = \frac{\text{Fresh weight of soil} - \text{dry weight of soil}}{\text{Dry weight of soil}} \times 100$$

The soil filled in the pots was analyzed for phosphorous content according to the method of Soltanpur & Workman (1979). Lanyon & Heald (1982) method was used for the estimation of exchangeable Na<sup>1+</sup>,

K<sup>1+</sup> and, Ca<sup>+2</sup>, Mg<sup>2+</sup>, whereas Zn<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup> and Co<sup>3+</sup>, Cr<sup>3+</sup> were extracted with a solution of DTPA as described by Lindsay & Norvell (1978) using atomic absorption spectrophotometer. Soil organic matter was determined by the method of Black (1965).

Leaf relative water content (LRWC) was estimated by determining the turgid weight of 0.5 g fresh leaf samples by keeping them in water for 4 h, followed by drying in hot air oven till constant weight using the formula given by Gao (2000).

$$\text{RWC (\%)} = [(W-DW) / (TW-DW)] \times 100$$

W represents sample fresh weight; TW represents sample turgid weight; DW represents sample dry weight.

**Determination of leaf soluble protein, proline and chlorophylls:** Protein content of leaves was determined following the method of Lowry *et al.*, (1951) using BSA as standard. Proline content of leaves was estimated by the method of Bates *et al.*, (1973). The chlorophyll estimation of leaves was made following the method of Arnon (1949) as modified by Kirk (1968).

**Determination of quality indices of seed:** Seed oil content was estimated by NMR (Nuclear Magnetic Resonance) test (Robertson & Morrison, 1979). The oil was extracted from seeds of plants of different treatments after harvest in petroleum ether (40-60°C) as described by

Anon., (1980). The oil acid value was determined according to Anon., (1997). For estimation of seed protein content, 100 mg seeds were grounded and digested in Kjeldhal digestion flask. The total seed protein was then determined following the method as described by Anon., (1982). Glucosinolate content of the oil was determined following the method of Smith *et al.*, (1985). For quantification of fatty acids, fatty acid methyl esters were prepared. The methyl esters of the fatty acids (0.5 µl) were analyzed in a gas chromatograph (Shimadzu QP 5050) equipped with a flame ionizing detector (FID) and a fused silica capillary column (MN FFAP (50 m x 0.32 mm i.d; film thickness 0.25 µm). Helium was utilized as carrier gas. The column temperature was maintained at 110°C for 0.5 min, raised to 200°C at 10°C/min till 10 min. The temperatures of the injector and detector were set at 220°C and 250°C respectively.

**Statistical analysis:** The data was analyzed by two way ANOVA and comparison among mean values of treatments was made by Duncan's multiple range test (Duncan, 1955).

## Results and Discussion

Imposition of drought stress for 10 d reduced the soil moisture content from 22-9%. The nutrient status of the soil filled in the pots and used in the experiment is given in Table 1.

**Table 1. Macro and micronutrients (µg/g) analysis of soil filled in the pots used for seed germination and seedling growth of linseed.**

Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>1+</sup>	Na <sup>1+</sup>	Cu <sup>2+</sup>	Fe <sup>2+</sup>	Co <sup>3+</sup>	Zn <sup>2+</sup>	Cr <sup>3+</sup>	Total nitrogen (%)	Available phosphorous (mg/kg)	Soil organic matter (%)
3.8.00	1.19	53.34	11.21	2.50	16.22	1.79	3.12	1.00	0.046	5	6.2

Leaf relative water content (LRWC) is an important physiological attribute which determines the tolerance of plants to drought stress (Sánchez-Blanco *et al.*, 2002). It has a close relation with water potential of plants (Ober *et al.*, 2005). During the present study, LRWC was markedly decreased by drought stress in both the canola cultivars. The % decrease was significantly higher in cv. Dunkeld at p<0.05. Both under unstressed as well as drought stressed conditions; SA and Put were equally effective to ameliorate the effect of drought stress on LRWC, cv. Rainbow being more responsive (Fig. 1a). Leaf relative water content was decreased in the leaves of both drought tolerant and sensitive maize cultivars when subjected to low moisture levels (Valentovic *et al.*, 2006). Under unstressed condition both SA and Put were more effective. Exogenously applied SA ameliorated the adverse effects of drought stress on LRWC and hence economized the water budget of plants which was maintained well in cv. Rainbow. These results are in agreement with previous findings of Habibi (2012) who found that water content was higher in barley plants treated with SA under drought stress.

The chl *a* was significantly decreased by drought stress in both the cultivars. The % decrease was higher in cv. Rainbow. Salicylic acid significantly increased the chl

*a* content in cv. Dunkeld under unstressed condition. The growth regulators augmented the drought induced decrease in chl *a* content of both cvv. Rainbow and Dunkeld such that the chl *a* content was similar to that of untreated control. Under drought stress the affect of SA was more pronounced than that of Putrescine particularly in cv. Dunkeld the % increase was less. Drought stress did not significantly affect the chl *b* content of leaves in both the canola cultivars. Both the growth regulators non-significantly increased the chl *b* content; higher increase was observed in cv. Rainbow. The chl *a/b* ratio was higher in leaves of cv. Dunkeld. Drought stress caused a significant decrease in chl *a/b* ratio of both cvv. Rainbow and Dunkeld. Under drought stress, significantly higher reduction (47%) in chl *a/b* ratio was observed in cv. Rainbow. Under unstressed condition, SA and Put significantly decreased the Chl *a/b* ratio as compared with untreated control. Under drought stress conditions, both the growth regulators effectively ameliorated the drought induced decrease in chl *a/b* ratio (Fig. 1b, c, d). The leaf carotenoids were significantly affected by drought stress in cv. Rainbow. Both SA and Put were equally effective to alleviate the drought induced decrease and the carotenoids content did not differ significantly with control (Fig. 2a).

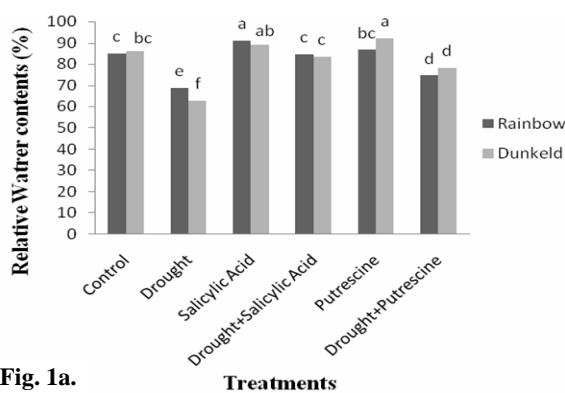


Fig. 1a.

Treatments

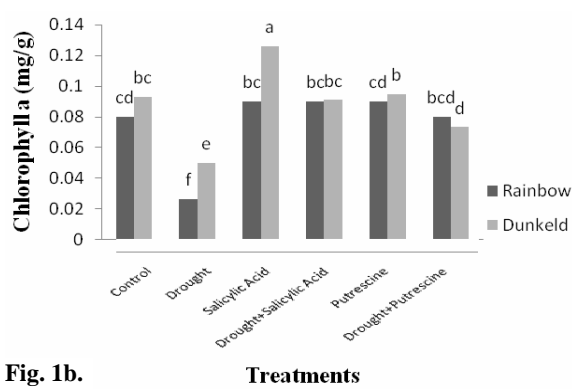


Fig. 1b.

Treatments

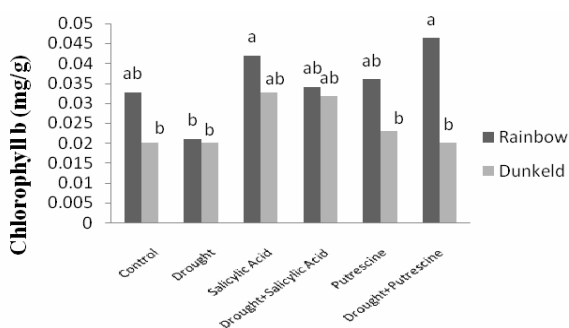


Fig. 1c.

Treatments

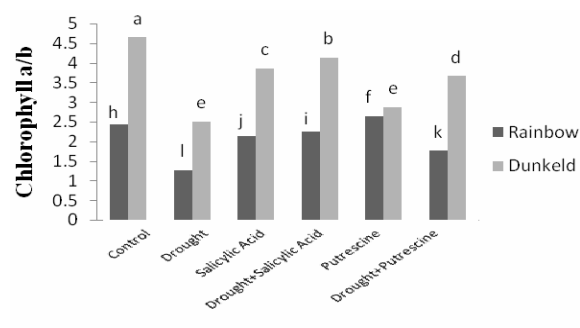


Fig. 1d.

Treatments

Fig. 1. a,b,c,d Effect of drought stress and plant growth regulators on leaf relative water content, chl *a*, *b* and chl *a/b* ratio in canola cvv. Rainbow and Dunkeld.

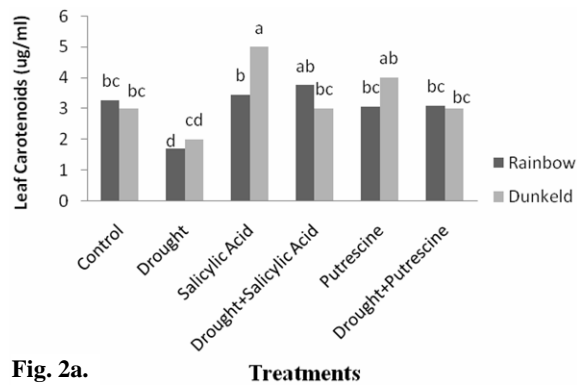


Fig. 2a.

Treatments

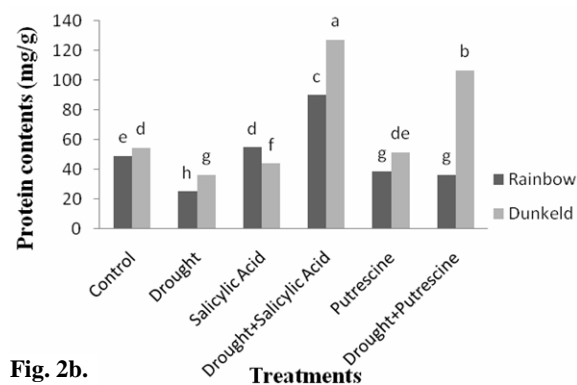


Fig. 2b.

Treatments

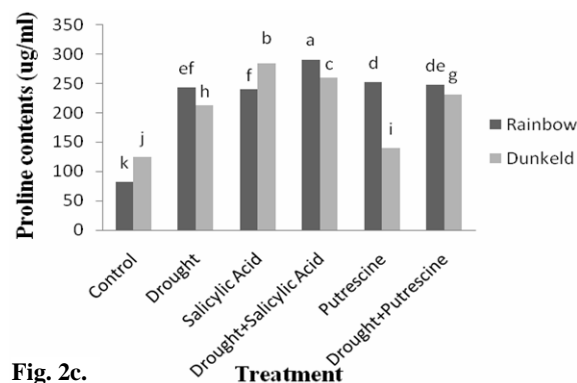


Fig. 2c.

Treatment

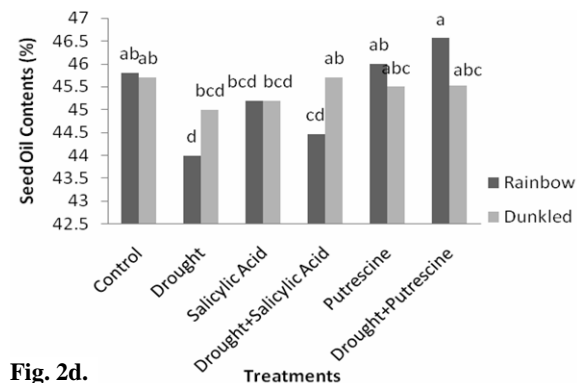


Fig. 2d.

Treatments

Fig. 2. a,b,c,d Effect of drought stress and plant growth regulators on leaf carotenoids, soluble proteins, proline and seed oil contents in canola cvv. Rainbow and Dunkeld.

Moisture stress cause reduction in leaf chlorophyll content of plants (Paknejad *et al.*, 2007; Sun *et al.*, 2011). Therefore, for better yields under stress, higher chlorophyll content might contribute to higher plant productivity (Rao *et al.*, 2012). The growth regulator SA augmented the drought induced reduction in chl *a*. The exogenous application of SA has been reported to regulate photosynthesis in plants grown under normal or stressful conditions (Abreu & Munne-Bosch, 2007; Korkmaz *et al.*, 2007). The chl *a/b* ratio is considered as an index of drought stress tolerance in plants and which was significantly lower in cv. Rainbow under drought stress. Sun *et al.*, (2011) reported decreased chl *a/b* in palm plants subjected to moisture stress.

The accumulation of soluble proteins and osmolytes such as proline serves as a means of osmotic adjustment which improves plant's tolerance to adverse effects of abiotic stresses (Ma & Turner, 2006). Soluble proteins were significantly higher in the leaves of cv. Dunkeld. Drought stress significantly decreased leaf soluble proteins by 50% in both cv. Rainbow and Dunkeld at  $p < 0.05$ . Under unstressed conditions, both Put and SA were less effective and were at par to control (untreated). Under drought stress, SA significantly increased the leaf soluble proteins in both the canola cultivars at  $p < 0.05$ ; cv. Dunkeld responded better (Fig. 2b). The observed increases in leaf protein due to SA and Put application under drought stress may demonstrate the role of these growth regulators in the induction of stress proteins. Zhong-zhu & Xiang-Wei (2004) have reported that exogenous application of SA ameliorated the adverse effects of drought stress on leaf soluble proteins in three shrub species. Similar was the case with Putrescine. These results are in agreement with previous findings of Saruhan *et al.*, (2006) who reported that exogenously applied polyamines enhanced the soluble proteins accumulation in the leaves of *Ctenanthe setosa* (Rosc.) Eichler under drought stress.

Drought stress induced accumulation of proline in canola leaves; maximum accumulation was recorded in cv. Rainbow. Exogenous application of growth regulators both under unstressed as well as drought stressed conditions significantly increased the proline accumulation at  $P < 0.05$  in both cv. Rainbow and Dunkeld. The response of cv. Rainbow to applied SA and Put was significantly greater under drought stressed condition (Fig. 2c). Similar results were also reported earlier (Umebese *et al.*, 2009). It is evident that foliar application of SA to canola plants under drought stress increased the accumulation of osmolytes like soluble proteins and proline in order to maintain LRWC which resulted in increased photosynthetic pigments under drought stressed conditions.

Drought stress did not affect the seed oil content in cv. Dunkeld. The cv. Rainbow showed a significant decrease in seed oil content upon the imposition of drought stress as compared with respective control. Under unstressed condition, SA and Put had their effect similar to control. Under drought stress SA was

ineffective to overcome the drought induced decrease in oil content but Put significantly increased the oil content of cv. Rainbow (Fig. 2d). The decrease in seed oil contents of oil seed crops under water stress is a common phenomenon (Ali *et al.*, 2009) as was also observed during current investigation. Seed oil content of ground nut was found to decrease under drought stress (Dwivedi *et al.*, 1996). The beneficial effects of Put on seed oil content under drought stress have been reported by El-Lethy *et al.*, (2010) in flax plants.

Drought stress has no significant affect on the seed proteins in canola. Salicylic acid significantly increased (37%) the seed proteins under drought stress in cv. Dunkeld (Fig. 3a). The oil/protein ratio was not significantly affected by drought stress and applied growth regulators. Putrescine showed slight decrease in oil/protein ratio of cv. Dunkeld as compared with respective untreated control (Fig. 3d). The polyamines were reported to affect oil composition of seeds during their effect on activating the synthesis of some enzymes involved in fatty acids metabolism (Talaat & EL-Din, 2005) and have been investigated to trigger protein synthesis and therefore, influence activities of enzymes involved in fatty acid synthesis (Serafini-Fracassini, 1991; Brown *et al.*, 1991).

Figure 3b showed that drought stress did not significantly affect seed moisture content in canola. In cv. Rainbow, SA significantly decreased the seed moisture under unstressed condition. The affect of growth regulators on seed moisture was non-significant at  $p < 0.05$ .

The glucosinolate contents were higher in the seed oil of cv. Dunkeld. Imposition of drought stress significantly increased the glucosinolates in seed oil of both cv. Rainbow and Dunkeld. Under unstressed conditions, the foliar application of SA caused accumulation of glucosinolates in seed oil. Its affect was reversed under drought stress showing a significant reduction in oil glucosinolates as compared with drought treatment alone. Under unstressed conditions, Put significantly decreased (13%) the oil glucosinolates of cv. Dunkeld as compared with untreated control. In contrast, cv. Rainbow showed accumulation of glucosinolates in response to Put application under unstressed condition. Put significantly decreased (52%) the drought induced accumulation of glucosinolates in cv. Dunkeld but not in cv. Rainbow as compared with drought treatment made alone (Fig. 3c). Glucosinolates are sulphur containing compounds which impart pungent smell to oil of mustard and rape. The accumulation of glucosinolates under drought stress imposed during flowering has been reported earlier by Bouchereau *et al.*, (1996) in rapeseed. During the present investigation, SA treatment decreased the drought-induced accumulation of glucosinolates. Under unstressed conditions, application of SA is reported to increase the concentration of glucosinolates in canola seeds (Kiddle *et al.*, 1994) as was also observed during current investigation.

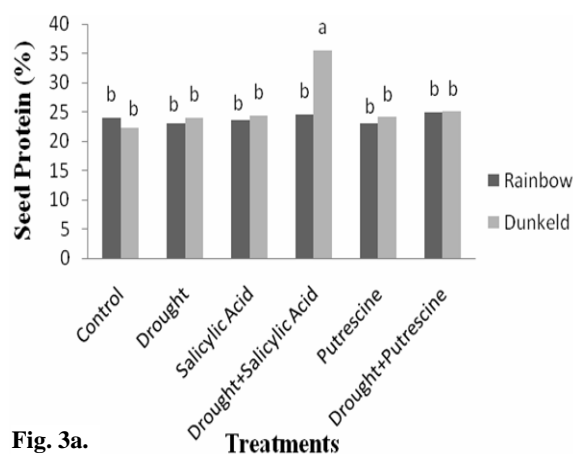


Fig. 3a.

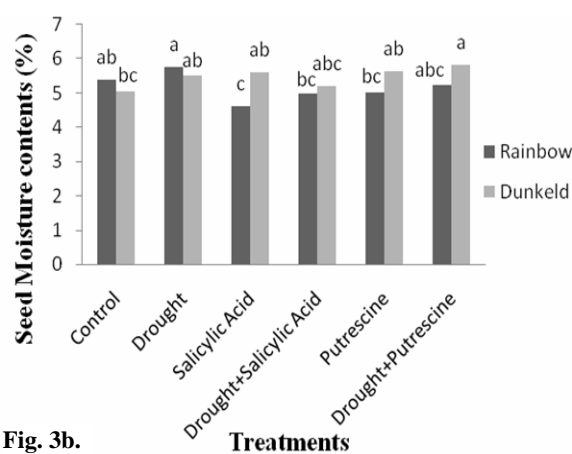


Fig. 3b.

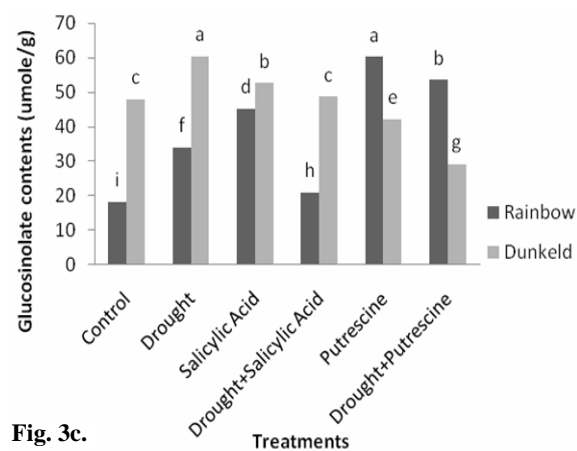


Fig. 3c.

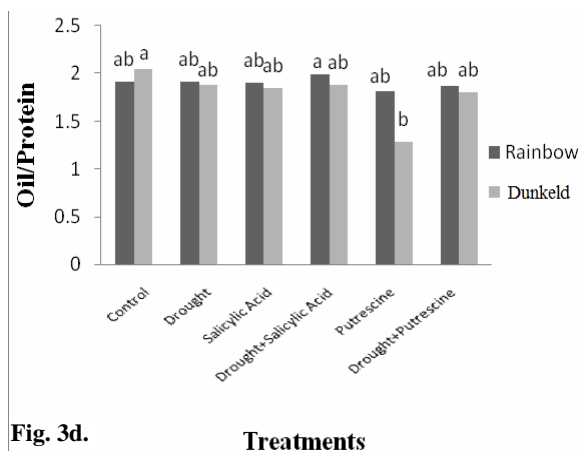


Fig. 3d.

Fig. 3. a,b,c,d Effect of drought stress and plant growth regulators on seed proteins, seed moisture, glucosinolates contents and oil/protein ratio in canola cvv. Rainbow and Dunkeld.

The basic level of erucic acid (C22:1) was higher in the seed oil of cv. Rainbow. Both cvv. Rainbow and Dunkeld showed increase in the level of erucic acid (C22:1) under drought stress. The response of two canola cultivars was different to applied growth regulators. Salicylic acid under unstressed as well as drought stressed conditions significantly decreased the oil erucic acid (C22:1) in cv. Rainbow. Its effect was more pronounced under drought stress condition. Putrescine was ineffective both under unstressed as well as drought stressed conditions. In contrast, cv. Dunkeld showed stimulatory response to applied Put and showed significant increase in oil erucic acid (C22:1) under drought stress condition. Imposition of drought stress significantly decreased the oil oleic acid (C18:1) content in both the canola cultivars. Under unstressed condition, growth regulators did not affect the oleic acid (C18:1) as compared with untreated control. Under drought stress, both the growth regulators significantly improved the oil oleic acid (C18:1) content at  $P < 0.05$  over that of drought treatment. The basal level of linoleic acid (C18:2) was similar in seed oil of both the canola cultivars. Drought stress as well as applied growth regulators did not significantly affect the oil linoleic acid (C18:2) in both the canola cultivars. Drought stress did not significantly affect oleic/linoleic ratio in canola seed oil (Fig. 4a, b, c, d). The acid value was significantly lower in the seed oil of cv. Rainbow. Drought stress as

well as growth regulators did not affect the oil acid value of cv. Rainbow. The oil acid value of cv. Dunkeld was significantly increased under drought stress at  $p < 0.05$ . Salicylic acid and Put significantly decreased the acid value of seed oil of cv. Dunkeld both under unstressed as well as drought stressed conditions (Fig. 5a).

Oleic/linoleic acid ratio is considered as an important indicator of stability and shelf life of vegetable oil (Worthington & Hammons, 1977). During current findings, the oleic/linoleic acid ratio was not altered by drought stress. Similar results were obtained by Unger (1982) that water stress resulted in a very little differences in oleic/linoleic acid ratios. Put improved the oleic/linoleic acid ratio under unstressed conditions. Salicylic acid markedly increased the content of oleic acid in seed oil of both cultivars under drought stress; probably due to the effect of this growth regulator on enzymes involved in fatty acid unsaturation. Sobrino *et al.*, (2003) found a close relationship between oleic acid and linoleic acid contents and concluded that the enzyme  $\Delta 12$  desaturase is responsible for such kind of responses. The elongation of carbon chain and number of double bonds play crucial role in determining the physical characteristics of both fatty acids and triglycerides (Mittelbach & Remschmidt, 2004). Erucic acid (C22:1) was reported to cause heart lesions in experimental

animals which were closely associated with heart diseases (Engfeldt & Brunius, 1975), further it imparts pungent smell to oil. During current investigation erucic acid (C22:1) was increased under drought stress whereas, marked decreases were observed in the content of oleic

acid (C18:1). This supported the previous findings of Bouchereau *et al.*, (1996) who found that drought induced decrease in oleic acid of rapeseed was associated with an increase in erucic acid content which were ameliorated by applied growth regulators.

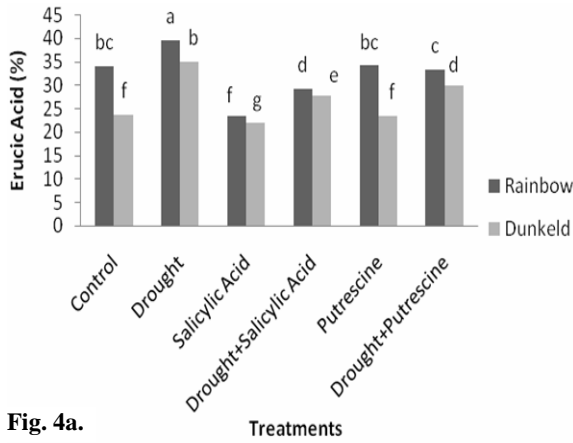


Fig. 4a.

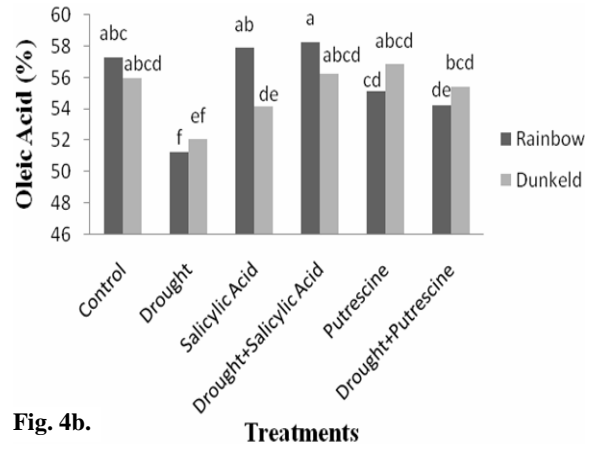


Fig. 4b.

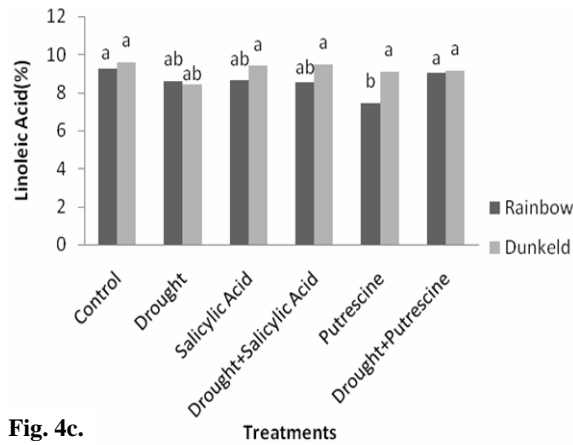


Fig. 4c.

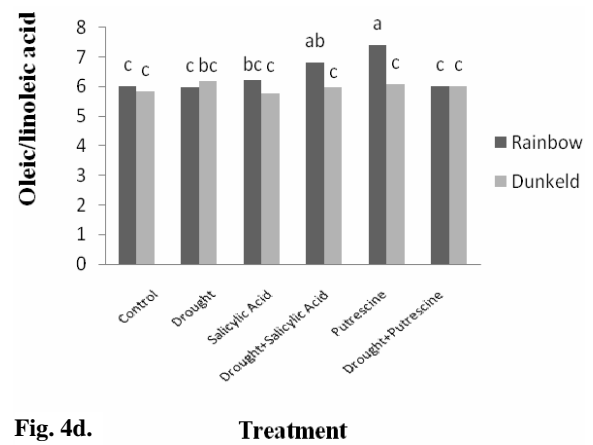


Fig. 4d.

Fig. 4. a,b,c,d Effect of drought stress and plant growth regulators on oil erucic acid, oleic acid, linoleic acid and oleic/linoleic acid ratio in canola cvv. Rainbow and Dunkeld.

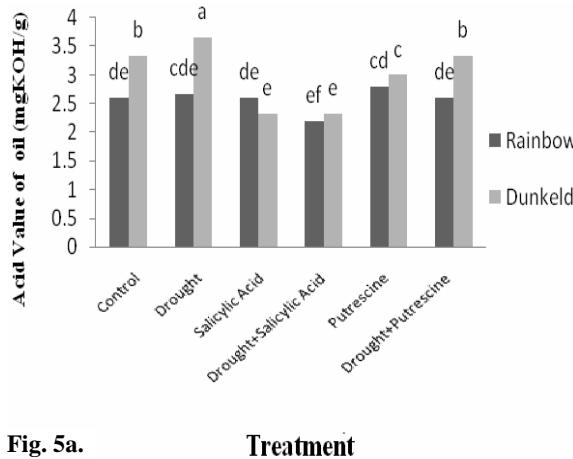


Fig. 5a.

Fig. 5. a Effect of drought stress and plant growth regulators on oil acid value in canola cvv. Rainbow and Dunkeld.

**Conclusion**

Drought stress imposed during flowering reduces the oil quality of canola by increasing the glucosinolates and erucic acid contents. There was also observed an increase in oil acid value and reduction in oleic acid content. The growth regulator SA was highly effective in augmenting the adverse effects of drought stress on oil quality indices in canola. On the other hand Put improved the seed oil contents under drought stress. Moreover, the SA also improved seed proteins under drought stress. The effect of SA appears to be mediated by improving photosynthetic pigments consequently enhancing photosynthesis, economizing the water budget as obvious by increased LRWC and by augmenting osmoregulant proline production and preventing proteins from getting degraded. It is inferred that SA which is economical and environment friendly alternative can be implicated to improve seed oil content and oil quality of canola in current scenario of drought and climate change.

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