The Journal of Animal & Plant Sciences, 29(3): 2019, Page: 730-736
ISSN: 1018-7081

SEED PRIMING ENHANCES CANOLA (*BRASSICA NAPUS* L.)YIELD BY MODULATING BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES UNDER WATER DEFICIT CONDITION

K. Khanum[1], S. A. Majid[1], A. Bibi[*1,2], A. Ulfat[1] and S. Ashraf[1]

[1]Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad -13100 Pakistan; [2]Department of
[2]Department of Botany, Women University of Azad Jammu and Kashmir, Bagh -12500 Pakistan
[*]Corresponding Author: wellwisherajk@hotmail.com

ABSTRACT

Canola (*Brassica napus* L.) is exposed to many environmental pressures that limit its development and yield. Hormonal seed priming is used to cope with adverse effects of stresses. Recent study was conducted to observe the influence of deficiency of water on growth and other developmental aspects of canola. Experiment was designed for two consective years i.e. 2014-15 and 2015-16 in Randomized complete block design with three replicates. Two rapeseed varieties after priming with different phytohormones viz. Indole acetic acid (IAA),Gibberellic acid (GA), Salicylic acid (SA) and Nitric oxid (NO) were used in 10^{-4} and 10^{-5}M concentration to study their role in mitigating stress effects. Total soluble sugars, protein and proline accumulation was found to increase under drought stress. Antioxidant enzymes showed varied response, while decrease in relative water contents and cell membrane thermostability was observed due to drought. Hormonal seed soaking was found to somewhat maintain biochemistry and physiology of Canola even under drought stress. Hence it is concluded that hormonal seed soaking is a best strategy to decrease the effects of stress in canola.

Key words: Canola, Seed priming, Drought, Phytohormones, Catalase, Protein

INTRODUCTION

In the world, Canola (*Brassica napus* L.) is one of the very important oil crops (Bybordi, 2010).Its oil is opulent in energy as 100 g of canola oil provides 884 calories. Canola is the world's third most major source of vegetable oil after soybean and palm (Shekari *et al*., 2015). There are different stresses that limit growth and yield of canola. Among different abiotic stresses, drought is more common that inhibit development and production of vegetables (Yamaguchi-Shinozaki *et al*., 2002). Drought cause reduction in quality and yield of many important crop plants (Hongbo *et al*., 2005). It disturbs all attribute of plant biochemistry and physiology during both vegetative and reproductive stages ultimately cause decline in yield (Moghadam *et al*., 2011). Canola is an economically significant crop of Pakistan but irregular rainfall and shortage of water for irrigation cause reduction in its yield and quality (Ghobadi *et al*., 2006).

Seed priming sets in motion activities involved in seed germination, such as respiration, endosperm weakening and seed reserve (starch) degradation, which facilitate the transition of quiescent dry seeds into germinating state and increase the germination potential and imposes abiotic stress to germinating seeds to stimulate stress responses (e.g., activation of ROS scavenging systems and accumulation of stress response proteins), hence inducing cross- tolerance (Li *et al*., 2013; Chen and Arora 2012). Seed priming can modify performance of Brassica seeds under drought stress in terms of germination and water use efficiency (Ajouri *et al*., 2004). During drought, priming caused quick seedling emergence, early flowering and increased grain yield (Kaur *et al*., 2005). Hormones play important role in plants by regulating their developmental and network signaling processes during abiotic stresses. Recent studies have revealed the ability of phytohormones in decreasing or eliminating the harmful effects of abiotic stresses (Khan *et al*., 2013).Many regions of Pakistan has the great potential for canola seed production however due to present global climatic variations the growth stage that need sufficient water supply for best growth and yield might have to face water deficiency which ultimately decrease its growth and production. So, present project was planned to improve the cultivation and production of canola in Pakistan by investigating the drought stress effect on biochemical and physiological attributes of canola and their remediation by phytohormones.

MATERIALS AND METHODS

Seeds of two varieties of *Brassica napus* L. HOYLA 433 and PARC were procured from Pakistan oilseed development board Islamabad, Pakistan. A total of five treatments i.e. Control, GA, SA, IAA and NO at 10^{-4}M and 10^{-5}M concentration were applied to the seeds and continuous aeration were provided by using aquarium pumps. Seeds after relevant treatments were grown in field according to four factorial randomized complete block design (RCBD) with three replicates during two consecutive years i.e. 2014-15 and 2015-16.

Flag leaves were sampled for biochemical and physiological analysis. This stage is particularly important because flag leaf contributes 70%in photosynthesis. Dubois *et al*. (1956) method was used to measure the flag leaves sugar content. Protein content was estimated by Bradford method (1976). Catalase activity was assayed according to Aebi (1984). Peroxidase activity was determined following the procedure used by Tutschek (1979). Proline content of flag leaves was assessed according to Bates *et al*. (1973). Cell membrane thermostability was measured following the method and formulae of Blum and Ebercon (1981). Relative water content (RWC) of leaf was determined according to Whettherley (1950)**.** Relative water content = [Fresh weight–dry weight/turgid weight–dry weight] × 100. Level of Significance for the variations of means was tested using four factorial ANOVA. Duncan's multiple range test (DMRT) at ($p \leq 0.05$)was used to compare the variance between means of the treatments (Steel *et al.,* 1997).

RESULT AND DISCUSSION

Protein contents: Protein contents revealed significant differences by stress and priming. Though the two years data was found non significantly different(Fig. 1). During both years, maximum protein was found in 10-4 M GA treated HOYLA 433 followed by 10^{-5}M NO primed PARC in drought condition. Direct alterations due to maximum temperature include aggregation and denaturation of protein, therefore protein content declined in heat stress. In current study protein contents were remained unaffected during year 2014-15 while decreased under stress during 2015-16. During deficiency of water, soluble protein was decreased due to a severe reduction in rate of Photosynthesis. Material that is necessary for synthesis of protein were not properly provided, as a result, protein synthesis was shortened noticeably or even stopped. Decline of protein content may be due to inhibition of protein synthesis or protein degradation (Howarth, 2005).

Total soluble sugar: Analysis of variance revealed non significant differences between two years results. During first study year under drought condition maximum sugar was found in 10^{-4} M SA treated HOYLA 433 while minimum was found in HOYLA 433 treated with 10^{-5} M NO. During 2015-16 maximum sugar was found in HOYLA 433 treated with 10^{-5}M IAA while minimum was found in10^{-5}M NO treated PARC under stress condition. In Recent study, total soluble sugars showed different behavior under different priming treatments. In young branch of common bean plants total soluble sugars content considerably increased under water deficient alone or in combination with H_2O_2 pretreatment. The rise in sugar quantity may be results of starch degradation. Starch reduction in leaves of grapevine was decreased due to shortage of water. In water-deficit plants the tolerance mechanism may be related with addition of osmoprotectants like soluble sugars. The deposit of soluble sugars is intensely associated to getting hold of drought tolerance in plants (Hoekstra and Buitink, 2001).

Ascorbate activity: Significant variations in ascorbate activity were observed by stress and priming. Though the data of two years i.e. 2014-15 and 2015-16 was found non significantly different (Fig. 3). Interaction of hormonal concentrations and varieties showed maximum ascorbate activity from PARC primed with 10^{-4} M SA while minimum was found in unprimed HOYLA 433 under drought condition. Water stress is certainly related with increased of oxidative stress in response to increased ROS accumulation, mostly$H_2O_2$and O_2^-in chloroplasts, mitochondria and peroxisomes. As a result, the stimulation of antioxidant activities of enzyme is a common adaptation strategy which is used by plants to overcome oxidative stresses (Foyer and Noctor, 2003).

Catalase activity: Two years data i.e. 2014-15 and 2015-16 for catalase activity was found non significantly different however significant variations in ascorbate activity were observed by stress and priming (Fig. 4). Interaction of varieties and varied hormonal concentrations revealed maximum catalase activity in 10^{-4} M treated PARC while minimum activity was observed in HOYLA 433 under control condition during 2014-15. During 2015-16 maximum catalase activity was found in PARC treated with 10^{-4} MGA under stress condition. Din *et al*. (2011) worked on priming treatments and reported decrease in adverse effects of reactive oxygen species by seed priming due to increased antioxidant activity. Similar finding were described by Ella *et al*.(2011) who detected improvement in emergence and growth of primed rice, wheat and maize seedlings due to increased activity of CAT and POD compared to unprimed seeds.

Proline accumulation: Proline contents revealed significant variations by drought stress and priming, though comparison between two years data was found non significantly different(Fig. 5).Minimum proline contents were observed in HOYLA 433 with 10^{-5} M GA priming while maximum proline were revealed by unprimed PARC in non-stress condition. Literature supports that shortage of water improved concentration of proline in all the canola cultivars; but, this increase was found different from different varieties. Increase the concentration of proline in conditions of stress is used as an adaptive tool by numerous plant species (Bakht*et al*., 2013; Hayat *et al*., 2013).

Cell membrane thermostability: Two years data i.e. 2014-15 and 2015-16 for cell membrane thermostability was found non significantly different however significant variations were observed by stress and priming (Fig 6). Maximum CMTS was found in HOYLA 433 treated with 10^{-4}M IAA followed by PARC treated with 10^{-5}M GA in stress condition while minimum proline was found in 10^{-4}M GA treated PARC under control condition. Baji *et al*. (2002) reported that plants can tolerate drought if they can maintain their membrane integrity and stability. Cell membrane stability was found to decline drastically under drought stress by Wang (2002). He worked on maize plants along with K applications and observed greater alteration in cell membrane thermostability by stress and K applications. This enhancement was generally ascribed the K role in rising osmotic adjustment capability and cell membrane stability.

Relative water contents: RWC from varieties and varied concentrations of hormones reveled significantly varied results (Fig. 7). Maximum RWC was found from PARC treated with 10^{-5}M IAA in drought condition while minimum was observed in HOYLA 433 under control condition. A reduce in relative water content (RWC) due to drought has been well-known in wide range of plants. Leaves of plants show vast decreases in RWC and water potential on subjection to drought. Performance of plants to deficiency of water significantly diminish relative water content, leaf water potential and rate of transpiration with raise in leaf temperature (Veselova *et al*., 2010).

Grain yield: Plant growth regulators viz. GA, SA, NO and IAA were used to prime the seeds of Brassica napus to study the drought stress induced variations. Grain yield were found significantly varied when drought stress imposed as compared to the plant did not faced drought (Fig 8). During 2014-15 maximum mean grain yield was found from non primed HOYLA 433 whereas, minimum grain yield was observed from HOYLA 433 treated with 10^{-4} M hormonal concentration. During 2015-16, maximum grain yield was revealed by PARC in non stress condition from primed samples with 10^{-4}M concentration. Under stress condition maximum grain yield was found from non primed PARC while minimum grain yield was found from Hoyla 433 treated with 10^{-4}M GA.

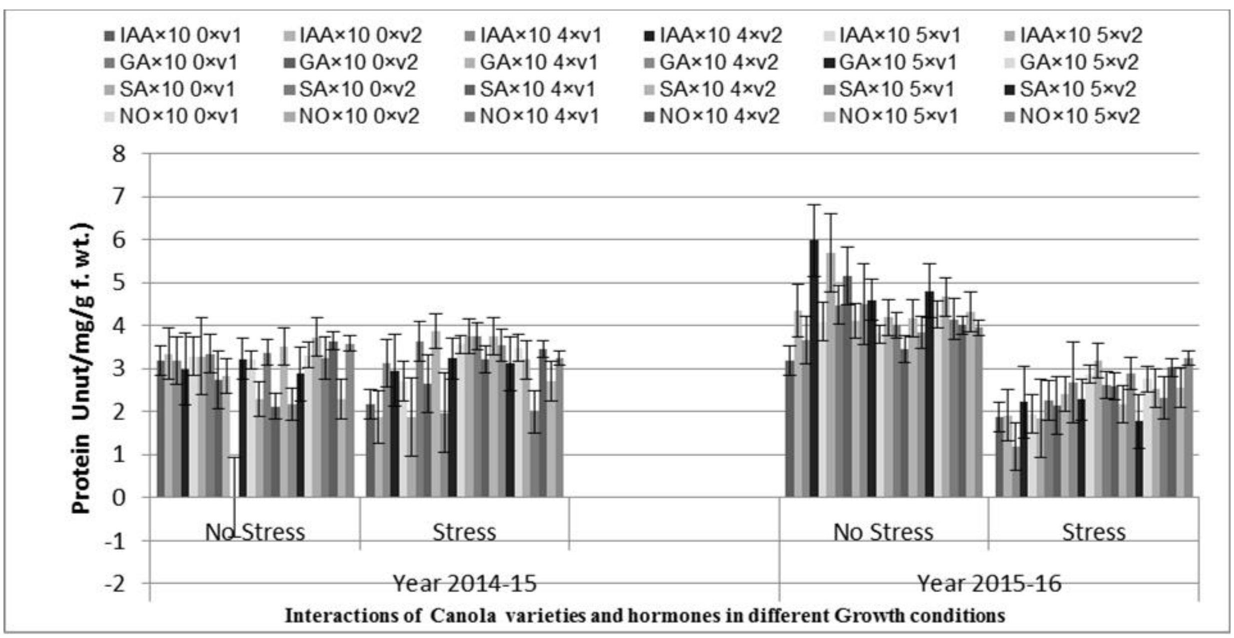

Figure 1: protein contents in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid(IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HOYLA 433(V1) and PARC (V2)

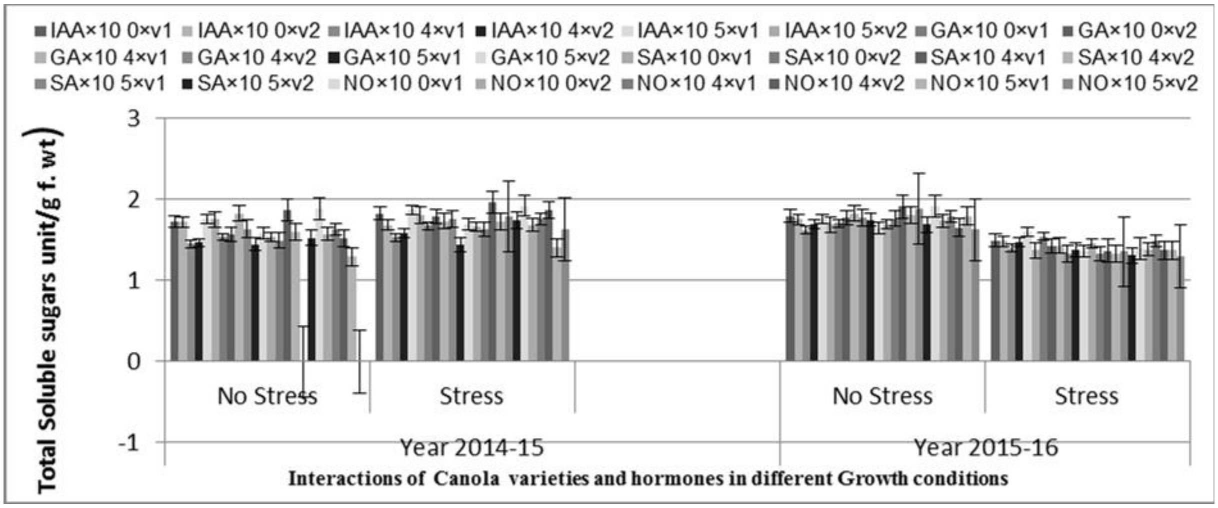

Fig 2: Total soluble sugars in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid (IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2)

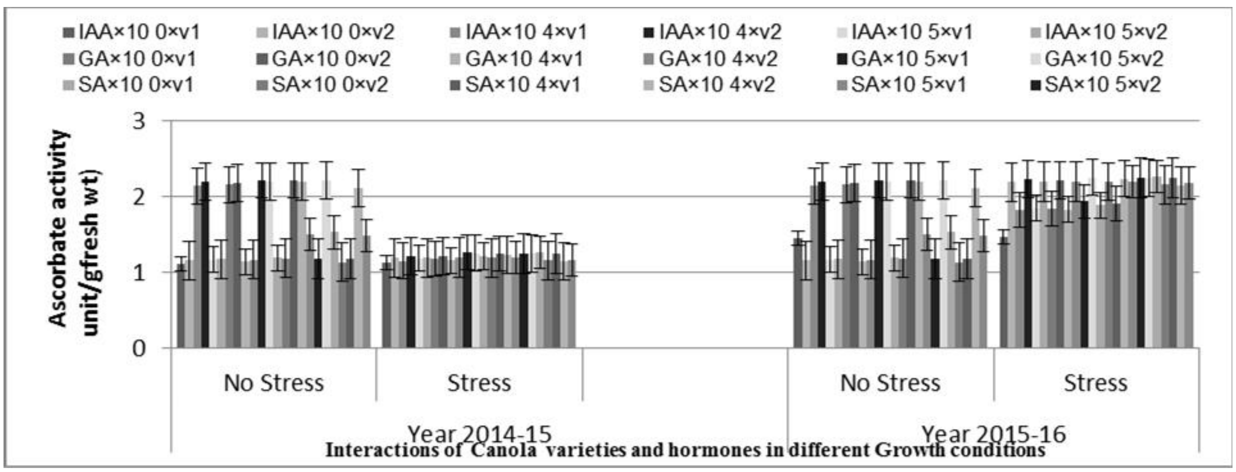

Fig 3: Ascorbate activity in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid (IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2)

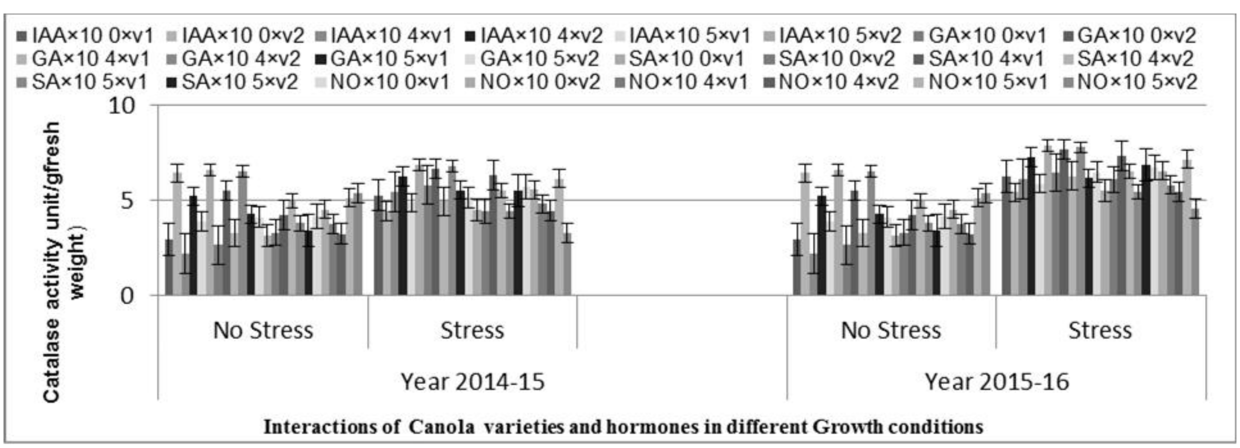

Fig 4: Catalase activity in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid(IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2)

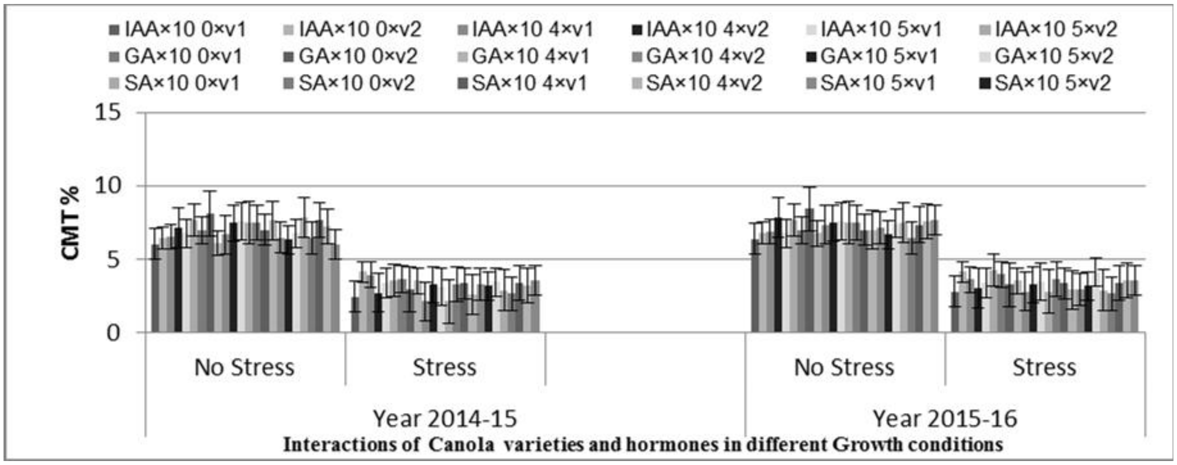

Fig 5: Proline accumulation in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid(IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2).

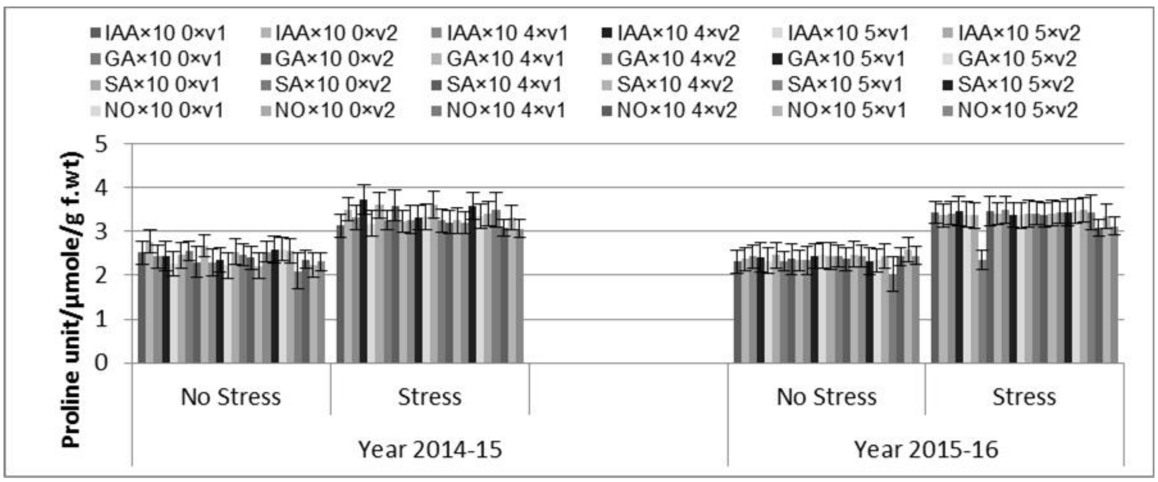

Fig 6: CMT% in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid(IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2)

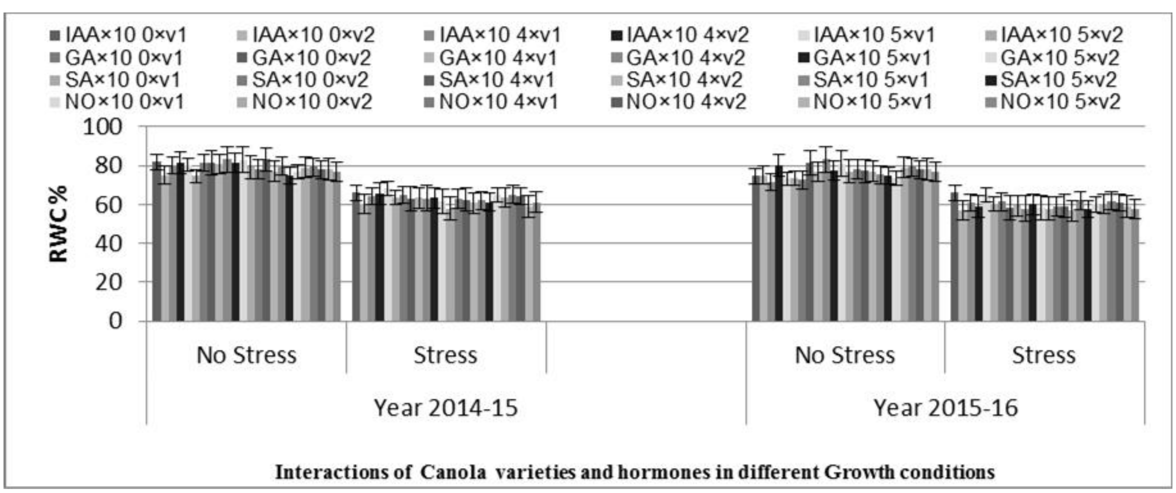

Fig 7: RWC% in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid(IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2).

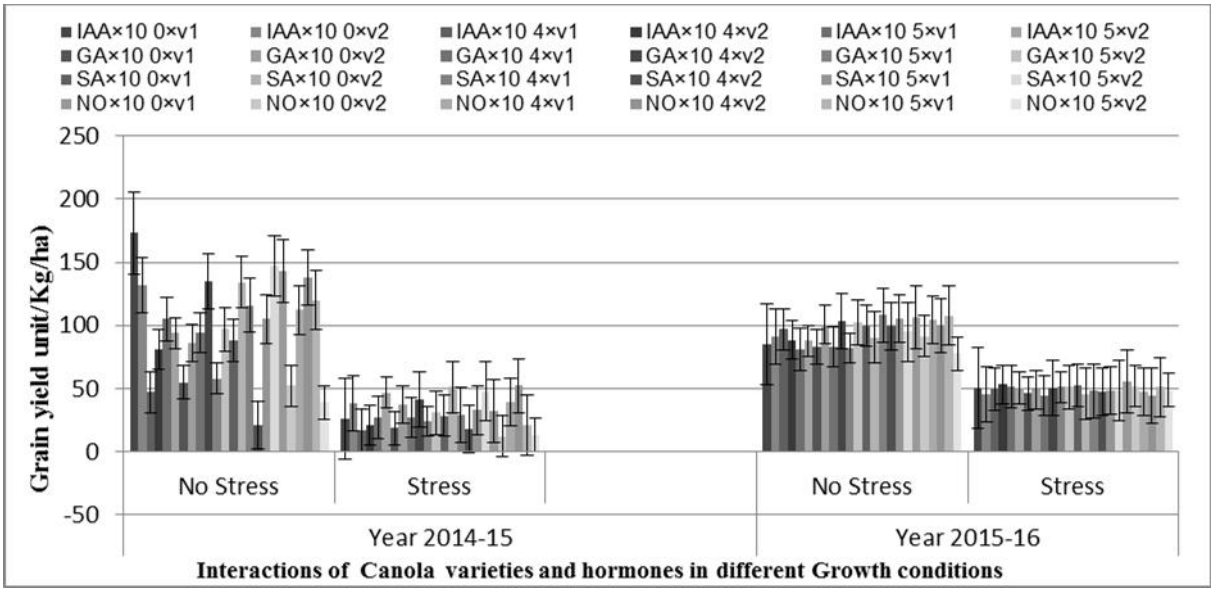

Fig 8: Grain yield in canola varieties during 2014-2016 under stress and no stress along with Indole acetic acid(IAA) Gibberellic acid (GA) Salicylic acid(SA) and Nitric oxide(NO) priming, HYOLA 443(V1) and PARC (V2).

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