Canadian Journal of Pure and Applied Sciences
Vol. 11, No. 2, pp. 4205-4213, June 2017
Online ISSN: 1920-3853; Print ISSN: 1715-9997
Available online at www.cjpas.net

INFLUENCE OF 28-HOMOBRASSINOLIDE ON PHOTOCHEMICAL EFFICIENCY IN *BRASSICA JUNCEA* UNDER DUAL STRESS OF EXTREME TEMPERATURES AND SALT

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ABSTRACT

The plants of *Brassica juncea* L. cv. RLC-1 were supplemented with 28-homobrassinolide (28-homoBL), exposed to dual stress of low and high temperatures along with salinity in the controlled environment, showed inhibitory effect on growth, photosynthetic pigments and efficiency of PSII. 28-homoBL mitigated the noxious effect of dual stress on *B. juncea* plants by promoting chlorophyll pigments which ultimately enhanced the photosynthetic efficiency of PSII reaction centres (RC) significantly. Extreme temperature of 4 and 44°C deteriorated total carotenoids to significant levels as compared to control distilled water plants exposed to (180 mM) NaCl salt or temperatures (24, 4 and 44°C). Our results indicated that 28-homoBL redirects the photosynthetic pigments of developing *B. juncea* seedlings to modulate the photosystem II activity for supporting photochemical efficiency at above level under single or dual stress of extreme temperature and salt.

Keywords: *Brassica juncea*, temperature stress, salt stress, 28-homobrassinolide, photosynthesis, photosystem II.

Abbreviations-28-homoBL, 28-homobrassinolide; DAS, days after sowing; PSII, photosystem II; NaCl, sodium chloride; ROS, reactive oxygen species; O^{2-}, superoxide radical; H_2O_2, hydrogen peroxide; OH^-, hydroxide radicals; $_1O^2$, singlet oxygen; $HgCl_2$, mercuric chloride; Chl a, chlorophyll a; Chl b, chlorophyll b; F_o, initial fluorescence; F_m, maximal fluorescence; F_v, variable fluorescence; PSII, Quantum yield of photosystem II; Y, Florescence yield; $_qP$, photochemical quenching; NPQ, non-photochemical quenching; ALA, 5-aminolaevulinic acid.

INTRODUCTION

Adversities in environmental conditions, such as air, suboptimal temperature and drought, undergoes daily fluctuations, erratic lows and highs and turn out to be damaging in few strokes, while others like salt and water stress may take days to become stressful (Taiz and Zeiger, 2006). Extreme temperatures both high/low are remarkable components that restrict the healthy growth and yield of plants (Houghton *et al*., 2001). The damage to plants exposed to extreme temperature stress has been reported to hinder photosynthesis, cell membrane injury, senescence and cell death (Xu *et al*., 2006). Probably, the cellular component most susceptible to temperature variation is the photosynthetic apparatus. Salvucci and Rafts-Brandne (2004) observed that photosystem II (PSII) and the CO_2 by Rubisco are the key sites of temperature stress on photosynthetic apparatus in plants. The primary effect of extreme temperature on the photosynthetic apparatus is the inhibition of the PSII activity and oxidative stress caused by the production of ROS (Yong *et al*., 2003). Meanwhile, ROS formed through stress are capable to damage cellular machinery including lipids by fatty acids, proteins through denaturation and unfolding, carbohydrate and nucleic acid (Blokhina *et al*., 2003). Uncontrolled production of ROS leads to oxidative stress can cause inhibition of the photosynthesis and respiration directed to retardation of plant growth and development (Jiang and Huang, 2001). Consequences of these extreme temperatures is the accumulation of various ions in plant system which burgeoned temperature effect to many folds by providing dual stress to the growing plants. Extreme temperature stress aggravates the adverse effects of stresses such as salinity responsible for limiting plant growth, development, productivity and quality of crops (Ashraf and Fooland, 2007). Salinity leads to imbalance in osmosis and ions which results in reduction of growth of crops leading to disturbance of both physiological and biochemical practices such as photosynthesis by plants, synthesis of proteins and lipid metabolism (Parida and Das, 2005).

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Various genetic approaches are used to reduce the undesirable possessions of these stresses on the normal performance of plant life. Furthermore, exogenous application of plant growth regulators (PGRs) both natural and synthetic is widely acceptable now days in crops as a mean of production enhancement. Hayat *et al*. (2008) and Hasan *et al*. (2008) reported that growth regulators boost stress tolerance of plants like metal, water, salt and temperature stress. Brassinosteroids, a new class of phytohormones play important role in elongation of shoots, pollen tube growth, vascular bundle demarcation, winding of leaves, inhibition of root, synthesis of ethylene, regulation of gene expression, biosynthesis of proteins and nucleic acids, metabolism of carbohydrates in photosynthesis (Yu *et al*., 2004; Cao *et al*., 2005), and seeking attention in studies like environmental stresses, such as drought stress (Zhang *et al*., 2008), metal stress (Ali *et al*., 2008), high temperature stress, salinity stress (Ali e*t al*., 2007), and oxidative stress (Cao *et al*., 2005). Several studies were conducted to find the consequence of extreme temperature and salt stress alone, and few literatures investigating the collective effect of these two stresses, but they need the suitable literature on brassinosteroids on the combined effects of salt and temperature because both are interconnected, as salt accumulates due to evaporation caused by temperature stress. Therefore, the present study was designed to assess the role of 28-homobrassinolide, an isomer of brassinosteroids in the tolerance of *B. juncea* L. to extreme temperatures and salinity stress.

MATERIALS AND METHODS

Plant growth conditions

Seeds of *B. juncea* L. cv. 'RLC- 1' were procured from Punjab Agriculture University, Ludhiana, India. This is an improved variety of *Brassica* released recently for farmers of Punjab. 0.01% $HgCl_2$ surface sterilization treatment was given to these seeds and then washed 5-6 times with double distilled water (DDW) followed by 8h imbibitions of 28-homoBL (0, 10^{-9}M) procured from Sigma-Aldrich, USA. NaCl was used for salt treatment and 5ml of (180 mM) NaCl was given as salt treatment and same amount of distilled water was used as control to the seeded plants. The treated seeds were propagated in triplicate in plant growth chamber (Navyug India ISO 9001-2008) under controlled conditions of 24±2°C temperature, dark/light 16/8h photoperiod. Shocking treatment of 4 °C and 44°C temperature was given to 7 day old seedlings for 5 h daily for three consecutive days followed by 24 h recovery period and 10 day old seedlings were harvested for various observations.

Growth analysis

Growth was analysed in terms of shoot, root length, fresh and dry weight in 10 day old seedling raised under different treatments. The experiment was repeated thrice with five biological replicates and the data given here is average value of all the experiments.

Estimation of photosynthetic pigments

Total Chlorophyll, Chl a and Chl b and total carotenoids content in leaves were estimated by the method of Lichtenthaler *et al*. (1987). The optical density (OD) was taken at 470, 645, 663 nm by spectrophotometer (UV Mini 1240, Shimadzu) against 5 ml of chilled 80% acetone used as a blank.

Chlorophyll fluorescence measurement

Chlorophyll fluorescence emission from the surface of the leaves of intact plants was measured with an imaging pulse amplitude modulated fluorometer (IMAG-MAXI, Heinz Walz, Germany) and calculated by the method of Genty *et al*. (1989). The minimum (F_o) and maximum (Fm) Chl a fluorescence emission were assessed in leaves after 2 hrs of dark adaptation and maximum quantum efficiency of PSII photochemistry was calculated as $F_v/F_m= (F_m-F_o)/F_m$. F_v is a variable fluorescence calculated as Fv/F_m-F_o. Then, the leaves were continuously illuminated with a white actinic light, which was equivalent to the actual growth light of *B. juncea* L. in order to measured Fs (steady state) and Fm (maximal Chl a fluorescence level in the light adapted leaves). Non - photochemical quenching of fluorescence (NPQ) was calculated as $NPQ= (F_m-F'_m) F'_m$. Photochemical quenching (qP) was calculated as $(F'_m-F_s)/F'_v$.

STATISTICAL ANALYSIS

The statistical analysis was executed by one-way analysis of variance (ANOVA). Each data was the mean of three replicates mean ± SE (n = 5) and comparison of p-values < 0.05 were significant and different from control.

RESULTS AND DISCUSSION

Effect of 28-homoBL on growth with or without stress

Growth of any plant can be measured in many ways like increase in shoot, root length, accumulation of fresh, dry weights. The results presented here, demonstrated positive effect of 10^{-9}M concentration of 28-homoBL on shoot, root length as compared to control DDW seedlings. Exposure to 10^{-9} M 28-homoBL resulted in a significant increase in shoot, root length 34 % and 70 % of *B. juncea* seedlings. Extreme temperatures and salt at all concentration (4C, 44°C and 180 mM) decreased shoot length 17, 33 % and 17 % and root length 21% for both 4°C and 44°C and 14% for salt treatment as compared to CN DDW seedlings (Table 1). When 10^{-9}M 28-homoBL supplemented with different temperature and salt, then it helped in reducing the deleterious effect of dual stress by 13 and 8% at 4°C and 44°C temperature in shoot length and 46 and 43% in root length. In previous reports, we found that salt stress reported to decrease mitotic index in

Table 1. Effect of (0,10^{-9}M) concentrations of 28-homoBL on shoot length and root length of *B. juncea* L. under dual stress of extreme temperature (4 , 44°C) and NaCl salt (180 mM) in laboratory conditions.

Treatments	Shoot length (cm)	Root length (cm)
Control	3.83±0.44[a]	3.13±0.18[a]
4°C Temp	3.20±0.05[a]	2.50±0.001[a]
44°C Temp	2.58±0.21[a]	2.50±0.28[a]
180mM NaCl Salt	3.20±0.43[a]	2.70±0.15[a]
10^{-9}M 28-homoBL	5.16±0.16[ab]	5.33±0.44[ab]
10^{-9} M +180 mM + 4 °C	4.33±0.44[ab]	4.60±0.10[a]
10^{-9} M +180 mM + 44 °C	4.16±0.16[ab]	4.50±1.00[a]
F-ratio$_{6\times14}$	6.87	3.84
HSD	1.408	2.596

The data represented above are Mean, S. E ± (n=5). Different superscripted alphabetical letters (a, b, c) within a column indicate significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Table 2. Effect of (0,10^{-9}M) concentrations of 28-homoBL on fresh weight, dry weight and moisture content of *B. juncea* L. under dual stress of extreme temperature (4, 44°C) and NaCl salt (180 mM) in laboratory conditions.

Treatments	Fresh weight (mg)	Dry weight (mg)	Moisture content (%)
Control	12.96±1.04[a]	5.02±0.22[a]	7.94±1.05[a]
4 °C Temp	11.44±0.52[a]	4.48±0.21[a]	6.96±0.71[a]
44 °C Temp	10.81±0.76[a]	4.66±0.28[a]	6.14±0.97[a]
180mM NaCl Salt	12.45±0.26[a]	4.31±0.15[a]	8.13±0.36[a]
10^{-9}M 28-homoBL	20.80±0.65[b]	6.27±0.11[ab]	14.53±0.54[b]
10^{-9} M +180 mM + 4 °C	14.91±0.31[ab]	5.95±0.06[ab]	8.96±0.26[a]
10^{-9} M +180 mM + 44 °C	13.93±0.10[ab]	5.88±0.18[ab]	8.05±0.23[a]
F-ratio$_{6\times14}$	2.41	9.68	14.45
HSD	29.625	1.053	2.80

The data represented above are Mean, S. E ± (n=5). Different superscripted alphabetical letters (a, b, c) within a column indicate significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

barley seedlings Tabur and Demir (2009). Tajbakhsh *et al*. (2006) reported that higher salt concentrations delayed mitosis and caused aberrant anaphase in barley seeds. Temperature stress also reduced shoot length of tomato seedlings as reported by Rivero *et al.* (2004) and in *Phaseolus vulgaris* by Babu and Devaraj (2008). Preliminary work have demonstrated that 28-homoBL improved cadmium stress tolerance in rape leaves and also under variety of stresses viz. high temperature tolerance of brome grass (Wilen *et al*., 1995) and chilling stress (Fariduddin *et al*., 2011). Application of 24-epiBL mitigate the toxic effect of heat on wheat cells (Kulaeva *et al*., 1991) and brome grass cells (Wilen *et al*., 1995). Our earlier work also illustrated that BR defend plants from germination till ripeness by regulating various Asada Halliwell components (Geetika *et al*., 2014). BRs also improved the plant expansion after direct sowing in submerged paddy pots at low temperature (Bajguz and Hayat, 2009). The results are very much similar to those of Nassar (2004) who reported in *vitro* experiments of banana shoot culture that supplementation of 28-homoBL to the medium resulted considerable shoot elongation which was superior to control untreated cultures. BRs promoted shoot development in number of plant species as *Arabidopsis*, soyabean, mung bean, pea, rice and tomato (Mandava, 1988). In our results, root length is also increased under 10^{-9}M concentration of 28-homoBL. Mouchel *et al*. (2006), reported the connection between brassinosteroid biosynthesis and auxin signaling, which is required for optimal root growth, both embryonically and postembryonically. Thus, proper BR levels are required for the correct expression of many genes using the brx phenotype results from a root-specific deficiency of BR. Kartal *et al.* (2009) reported that exogenous application of BR also increases root growth and cell division, most probably changing both BR and auxin responsive genes.

Enhancement or reduction in overall weight of plant under changing environment is a direct indicator of plant physiological state and its rate of growth. Fresh weight of plant is directly related to water content which is further related to water uptake phenomenon of plant our results indicated that under extreme conditions of temperature especially 44°C maximum decrease 17 was found but when supplementation of 10^{-9} M concentrations of 28-

Table 3. Effect of (0,10^{-9}M) concentrations of 28-homoBL on chlorophyll *a* content and chlorophyll b content of *B. juncea* L. under dual stress of extreme temperature (4, 44 °C) and NaCl salt (180 mM) in laboratory conditions.

Treatments	**Chlorophyll *a* Content (µg g^{-1}FW)**	**Chlorophyll *b* Content (µg g^{-1}FW)**
Control	5.58±0.06^{a}	2.11±0.25^{ab}
4 °C Temp	4.22±0.15^{a}	1.53±0.06^{a}
44 °C Temp	4.03±0.33^{a}	0.80±0.08^{a}
180mM NaCl Salt	3.55±0.14^{a}	1.18±0.05^{a}
10^{-9}M 28-homoBL	11.27±0.90^{ab}	5.06±0.29^{b}
10^{-9} M +180 mM + 4 °C	8.28±0.22^{ab}	3.45±0.11^{ab}
10^{-9} M +180 mM + 44 °	7.92±0.69^{ab}	2.82±0.09^{ab}
F-ratio$_{6\times14}$	31.29	51.69
HSD	2.171	0.850

The data represented above are Mean, S. E ± (n=5). Different superscripted alphabetical letters (a, b, c) within a column indicate significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Table 4. Effect of (0,10^{-9}M) concentrations of 28-homoBL on total chlorophyll content and total carotenoids content of *B. juncea* L. under dual stress of extreme temperature (4, 44 °C) and NaCl salt (180 mM) in laboratory conditions.

Treatments	**Total chlorophyll content (µg g^{-1}FW)**	**Total carotenoids content (µg g^{-1}FW)**
Control	8.16±0.41^{a}	2.65±0.15^{ab}
4 °C Temp	5.78±0.43^{a}	1.70±0.14^{a}
44 °C Temp	6.68±0.09^{a}	1.69±0.11^{a}
180mM NaCl Salt	6.72±0.57^{a}	2.30±0.06^{a}
10^{-9}M 28-homoBL	15.53±0.54^{ab}	6.42±0.28^{bc}
10^{-9} M +180 mM + 4 °C	12.95±1.35^{ab}	4.56±0.16^{b}
10^{-9} M +180 mM + 44 °C	12.52±0.36^{ab}	3.77±0.10^{ab}
F-ratio$_{6\times14}$	34.99	120.84
HSD	2.823	0.720

The data represented above are Mean, S. E ± (n=5). Different superscripted alphabetical letters (a, b, c) within a column indicate significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

homoBL is given with temperature and stress then maximum increase 15% was found in seedlings treated with 4˚C temperature and 180 mM NaCl stress. Parallel to fresh weight, total dry weight was also showed dynamism in its level and 10^{-9}M 28-homoBL revealed significant increase in dry weight (6.27 mg) (Table 2). 10^{-9}M concentration of 28-homoBL in combination with dual stress revealed less effect on total dry weight 18 % and 17 % at 4^{o}C and 44^{o}C supplemented with 180 mM NaCl salt as compared to CN DDW. In case of moisture content, 10^{-9}M concentration of 28-homoBL revealed maximum enhancement in moisture content (8.96 mg) at 4^{o}C temperature and 180 mM NaCl treatments. Which is followed by 44°C temperature and salt where only 1% enhancement was observed as compared to CN DDW treated seedlings (Table 2). Zhang *et al*. (2008) demonstrated similar type of results in *G. max* where 0.1 mg L^{-1} treatment of BR enhanced biomass accumulation in plants. Spall *et al*. (2016) also explored the growth promoting potential of 28-homoBL in *Brassica oleracea* var. cauliflower, cabbage and broccoli. Use of 28-homoBL as seed priming treatment prepared the germinated seeds to tackle with upcomimg hitches in the way of growth may be by triggering the multifactorial signalling pathways involved interaction between BRs, Ethylene, GA (Bajguz and Hayat, 2009).

Effect of 28-homoBL on photosynthetic pigments with or without stress

In case of photosynthetic pigments, decrease in total chlorophyll content up to 30%, 19% and 18% was observed in seedlings of *B. juncea* exposed to 4, 44 °C and 180 mM NaCl salt stress (Table 4). 10^{-9}M concentration of 28-homoBL increased the chlorophyll content in dual stress of temperature and salt treated seedlings as compared to non treated seedlings and maximum increase 58% is found in 10^{-9}M 28-homoBL treated seedlings grown in 4°C temperature supplemented with 180 mM NaCl salt stress. Chlorophyll a content (Table 3) was decrease up to 37% when *B. juncea* seedlings without 28-homoBL priming treatment were grown; but, enhancement up to 48% is seen in 28-

Table 5. Effect of ($0,10^{-9}$M) concentrations of 28-homoBL on initial florescence (F_v/F_o) and efficiency of PS II (F_v/F_m) of *B. juncea* L. under dual stress of extreme temperature (4, 44 °C) and NaCl salt (180 mM) in laboratory conditions.

Treatments	Initial florescence (F_v/F_o)	Efficiency of PS II (F_v/F_m)
Control	0.25±0.02[a]	2.23±0.04[a]
4 °C Temp	0.17±0.008[a]	2.17±0.008[a]
44 °C Temp	0.11±0.001[a]	2.11±0.007[a]
180mM NaCl Salt	0.17±0.001[a]	2.19±0.01[a]
10^{-9}M 28-homoBL	0.43±0.02[ab]	3.98±0.07[ab]
10^{-9} M +180 mM + 4 °C	0.35±0.26[ab]	3.27±0.07[a]
10^{-9} M +180 mM + 44 °C	0.30±0.002[a]	3.01±0.04[a]
F-ratio$_{6\times14}$	1.583	1.217
HSD	0.400	0.449

The data represented above are Mean, S. E ± (n=5). Different superscripted alphabetical letters (a, b, c) within a column indicate significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Table 6. Effect of ($0,10^{-9}$M) concentrations of 28-homoBL on photochemical quenching (q_P), non-photochemical quenching (NPQ) and florescence yield (Y) of *B. juncea* L. under dual stress of extreme temperature (4, 44°C) and NaCl salt (180 mM) in laboratory conditions.

Treatments	Photochemical quenching (q_P)	Non-photochemical quenching (NPQ)	Florescence yield (Y)
Control	3.35±0.01[ab]	1.14±0.02[a]	3.73±0.01[a]
4 °C Temp	3.24±0.02[a]	2.09±0.06[ab]	3.18±0.005[a]
44 °C Temp	3.14±0.03[a]	2.16±0.01[ab]	3.10±0.004[a]
180mM NaCl Salt	3.29±0.02[a]	2.15±0.002[ab]	3.27±0.005[a]
10^{-9}M 28-homoBL	4.13±0.02[cd]	1.23±0.03[a]	5.71±0.003[ab]
10^{-9} M +180 mM + 4 °C	4.84±0.005[bc]	1.37±0.01[a]	4.58±0.002[ab]
10^{-9} M+180mM + 44 °C	4.78±0.01[b]	1.28±0.01[a]	4.54±0.005[ab]
F-ratio$_{6\times14}$	113.72	10.18	4.90
HSD	0.153	0.135	0.428

homoBL primed seedlings grown in 4°C temperature supplemented with 180 mM NaCl salt. This increase was 11% more as compared only 4°C temperature and 4% more than only 180 mM NaCl salt treated seedlings. Chlorophyll b content (Table 3) also increased in 28-homoBL treated seedling supplemented with 4°C, 44°C temperature and 180 mM NaCl salt stress and highest increase up to 139% has been seen in only 10^{-9}M treated seedlings grown in controlled conditions. These results are similar with those of Santos (2004), Akram and Ashraf (2011), Kaur *et al*. (2014) who performed number of experiments with sunflower callus and other plants and shown that the key precursor of Chlorophyll is glutamate and 5-aminolaevulinic acid (ALA), that is decreased in calli and leaves under stress conditions, which indicates that stress influenced more markedly Chl biosynthesis than Chl breakdown. This decrease in photosynthetic pigments is similar with the proposal that, photosynthesis is the first most susceptible physiological process inhibited by exposure to extreme temperature and salt stress (Berry and Bjorkman, 1980). Temperature directly influences the working of photosynthetic apparatus effectively by disturbing the functioning of carbon reduction cycle, electron transportation in thylakoid and stomatal functioning (Allen and Ort, 2001).

Carotenoid is an accessory pigment found in plants and essential part of antenna complex of photosynthetic machinery of plants. In our results, an increase in total carotenoids content upto 72% (Table 4) was observed in 4°C temperature stressed seedlings supplemented with 180 mM NaCl salt and pre-treated with 10^{-9}M 28-homoBL. This increase was 36% as compared to only 4 °C temperature and 58% as compared to only 180 mM NaCl salt and highest increase up to 142 % was observed in only 10^{-9}M treated seedlings. These results showed close proximity with Bajguz (2011) who found an increase in the level of total carotenoids in *Chlorella vulgaris* after application of 28-homoBL. Kaur *et al.* (2014, 2015, 2017) demonstrated the elevation of total carotenoids in indian mustard plants after pretreatment of 28-homoBL as compared to untreated control plants. Anuradha and Rao (2009) also reported increased carotenoid content in radish plant be BRs application. Total carotenoids decreased under salt and temperature treatments, this decrease in level of carotenoids such as α-

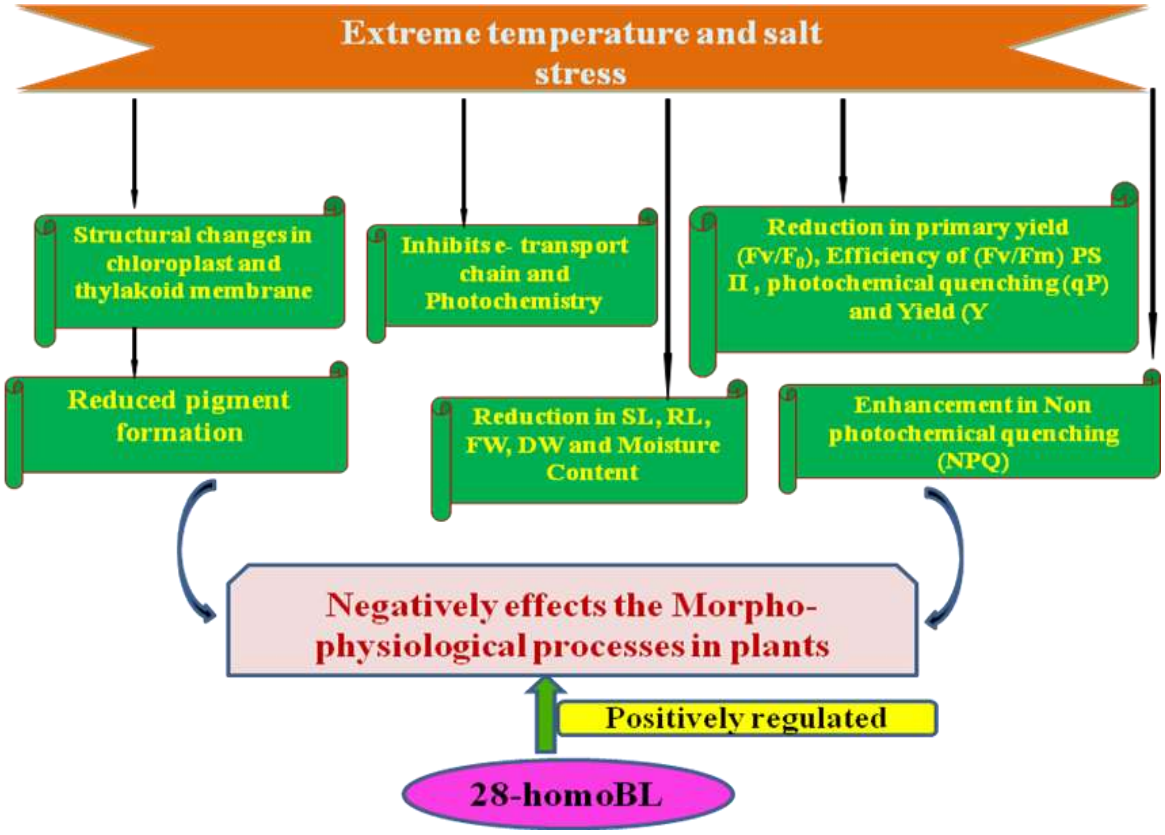

Fig. 1. Effect of extreme temperature and salt on morpho-physiological processes of plants.

carotene, β-carotene, and xanthin might abide the excitation energy of chlorophyll to transmit from photosynthetic reaction to outside which is one of the mechanism adopted by plants to protect chlorophyll to photo oxidation that lead to degradation in chlorophyll (Telfer, 2002).

Effect of 28-homoBL on chlorophyll fluorescence with or without stress

Photosystem II (PSII) plays a key role in response of leaf photosynthesis to environment perturbations (Baker, 1991; Dubey, 2005). As the key process of primary metabolism, extreme temperature and salt inhibition of photosynthesis may be due to stomatal closure (Ashraf and Harris, 2013). Initial florescence (F_v/F_o) and maximum florescence (F_v/F_m) in terms of photosynthetic efficiency is the frequently used indicator of photo inhibition of PSII in response to stress (Baker, 2008; Calatayud and Barreno, 2004). We observed that F_v/F_m decreased significantly under extreme temperature (4, 44ºC) and 180 mM NaCl (Table 5). However, it was significantly alleviated by treatment 10^{-9}M 28-homoBL. Earlier studies have shown that dual application of temperature and salt inhibits the PSII activity in *B. juncea* L. seedlings (Ahmad *et al*., 2012; Hayat *et al*., 2011). In the present study, our research is to know the potential mechanisms of this inhibited PSII activity and how 28-homoBL plays its active role in controlling the activity of PSII. In our results, maximum primary yield (F_v/F_o), Efficiency of (F_v/F_m) PS II photochemistry and photochemical quenching (qP) and Yield (Y) were markedly decreased by (32, 56, 32%), (3, 6, 2%), (14, 17, 13%) and (15,17% and 13%) respectively under 4, 44°C, and 180 mM NaCl salt stress in comparison to control plants but NPQ was significantly increased under 4, 44°C, and 180 mM NaCl, this increase was (83, 89 and 88%) respectively as compared to CN DDW treated seedlings (Table 6). 28-homoBL in combination with extreme temperature and salt showed significant increase in the chlorophyll florescence of photosystem II (Table 5-6). The results indicated that F_v/F_o, Fv/Fm, qP and Y were higher in alone 28-homoBL treated *B. juncea* seedlings, this enhancement was 72, 78, 10, 53 %, respectively. Injury to elements of thylakoid membranes, particularly those of PSII, and inhibition of energy transport from antenna molecules to reaction centres can lead to minimization of F_v/F_m ratios (Krause and Wies, 1984) as well as light inhibitory damage (Colom and Vazzana, 2003). Mathur *et al*. (2013) observed a turn down in F_v/F_m, PSII after introduction to extreme temperature and salt in *Triticum aestivum*. However, salt stress stimulated inhibition in plant is often ascribed to the reduced

photosynthetic performance (Wu *et al*., 2010; Akram and Ashraf, 2011). Correspondingly, Ashraf and Harris (2013) concluded that salinity, drought, and thermal stress can appreciably affect the performance of photosynthesis, stomatal manage to CO_2 delivery, transport of electrons in thylakoid membrane and carbon diminution cycle. In addition, our study evidently shown that combination of extreme temperature and salt significantly inhibits the quantum yield of PSII electron transportation and florescence yield in *B. juncea* L. seedlings as compared to only salt stressed seedlings. We also revealed that such a reduction in PSII was related to the alteration of qP and NPQ (Table 6). A reduction in photochemical quenching (qP) induced by dual stress of extreme temperature and salt showed closed PSII, which results in reduction of excitation energy used in photochemistry. The major alterations in PSII are decreased value of qP and F_v/F_m, as well as the increased value of NPQ (Genty *et al*., 1989). In our results, Non-photochemical quenching (NPQ) of *B. juncea* was decreased significantly by 7% in 10^{-9} M alone treated plants. However, 4 °C, 44 °C treated plants supplemented with 180 mM salt and pretreated with 28-homoBL showed further increase by 20% and 12% as compared to only temperature, salt and 10^{-9}M 28-homoBL treated plants alone. The reduction of PSII capacity may be due to oxidative stress (Zhou and Yu, 2006). Measuring the non-photochemical quenching and dissipation energy does not assent photon flux into dissipation process (P). Application of 28-homoBL results in increased photochemical quenching (qP) at the expense of non-photochemical quenching (NPQ), leading to less dissipation of excitation energy in the PSII antennae (Horton *et al*., 1996). 28-homoBL application protected the PSII against over excitation which could have caused damage, perhaps from a loss of integrity in the thylakoid membrane (Ogweno *et al*., 2010).

Non-photochemical quenching processes quench singlet excited chlorophylls and harmlessly dissipate excessive excitation energy as heat, thus helping to regulate and protect photosynthesis in response to excess light energy (Muller *et al*., 2001). Ahammed *et al*. (2012) also found that 24-epibrassinolide can decrease NPQ and activate photo-protection, in present study, 28-homoBL also increased the proportion of energy used in photochemical reactions (PSII), resulting in improved photosynthetic activity.

CONCLUSION

In conclusion, it was shown that temperature and salt stress lessen the growth by inhibiting photosynthesis (Fig. 1). This was ameliorated by the application of 10^{-9}M concentration of 28-homoBL, particularly through sustaining pigments, photochemical quenching capacity, maintaining electron excitation between photo system I (PSI) and photo system II (PSII). The results of this study not only present the physiological mechanisms of 28-homoBL induced temperature and salt tolerance, but also to provide prospective application in cultivation of crops.

ACKNOWLEDGEMENTS

Authors are highly acknowledged to Head, Department of Botany, Punjabi University Patiala, for providing laboratory facilities and UGC-New Delhi for financial assistance in major research project vide F. No. 42-920-2013(SR).

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Received: Feb 13, 2017; Accepted: March 10, 2017