Pol. J. Environ. Stud. Vol. 30, No. 4 (2021), 1-12
DOI: *10.15244/pjoes/128579* ONLINE PUBLICATION DATE:

Original Research

Penicillium Glabrum Acted as a Heat Stress Relieving Endophyte in Soybean and Sunflower

Ismail[1], Muhammad Hamayun[1]*, Anwar Hussain[1], Amjad Iqbal[2], Sumera Afzal Khan[3], Sarah Gul[4], Hamayoon Khan[5], Khushnood Ur Rehman[6], Hamida Bibi[7], In-Jung Lee[8]**

[1]Department of Botany, Abdul Wali Khan University Mardan, Mardan, Pakistan
[2]Department of Food Science & Technology, Abdul Wali Khan University Mardan, Mardan, Pakistan
[3]Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan
[4]Department of Biological Sciences, FBAS, International Islamic University, Islamabad, Pakistan
[5]Department of Agronomy, University of Agriculture Peshawar, Peshawar, Pakistan
[6]Department of Botany, Islamia College University Peshawar, Peshawar, Pakistan
[7]Department of Soil and Environmental Sciences, University of Agriculture Peshawar, Peshawar, Pakistan
[8]School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

Received: 28 July 2020
Accepted: 18 October 2020

Abstract

High temperature is one of the compelling ecological stresses faced by the plants owing to anthropogenic activities of mankind. Recently, endophytic fungi are considered to be a novel tool to counteract the consequences of thermal stress in crops. Owing to the problem, we isolated fungal endophytes from *Dryopteris blanfordii* and their growth favoring potential in rice seedlings. Endophytic fungi have the proficiency to secrete plant growth stimulating secondary metabolites that help the host plants to restore growth under harsh environments. The isolated *Penicillium glabrum* (DryR-30) helped the host plant species, i.e. *Glycine max* L. and *Helianthus annuus* L. under thermal stress. *P. glabrum* associated with *G. max* and *H. annuus* exposed to 40ºC not only boosted their host growth attributes (total biomass, plant height and chlorophyll contents), but also significantly curtailed the synthesis of reactive oxygen species and lipids peroxidation. Moreover, the abscisic acid (ABA) and proline concentrations were significantly reduced, while the accumulation of ROS-degrading enzymes, such as catalase (CAT), peroxidase (POD), ascorbic acid oxidase (AAO), glutathione reductase (GR) and superoxide dismutase (SOD) were increased in *P. glabrum*-associated crops. Additionally, the protein,

*e-mail: hamayun@awkum.edu.pk
**e-mail: ijlee@knu.ac.kr

lipid and sugar contents in tested plants were also improved. These stirring findings suggest that *P. glabrum* can be applied to food crops as a thermal stress alleviation tool.

Keywords: endophytic fungi, *P. glabrum*, *Dryopteris blanfordii*, thermal stress, reactive oxygen species, antioxidants

Introduction

Better diet and tenable nutrition, are the two vital needs of human for which man is in incessant fight, while climate change makes these goals more challenging for mankind. Heterogeneity in the edaphic and climatic environments of a complex habitat regulates the topographical dispersal outline of vegetations [1-4]. Plants growing under stress display some level of adaptability to stressful conditions [5-8]. At high temperature; loss in yield and other growth parameters of crop species are expected [9, 10]. Moreover, proteins, lipids and chlorophyll molecules can be degraded by the oxidative action of ROS (hydroxyl radical (OH), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and superoxide (O_2^{-1}) [5, 6, 11-14].

To evade oxidative losses, plants have self-activated-defense-mechanisms (SADMs), i.e. comprised of antioxidant systems and compatible osmolytes [15]. Another procedure adopted by plants, is the transport and storage of toxic molecules and ions to less sensitive tissues [16]. Antioxidant scheme adopted by plants, comprise enzymatic and non-enzymatic machineries that work together to detoxify ROS species. Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR) represent enzymatic-antioxidant system. On the other hand, ascorbic acid, tocopherol and some secondary metabolites symbolize the non-enzymatic-antioxidant system of plants [17].

Different biotic and abiotic stress causes accumulation of proline and phenolic compounds in higher plants. Proline and phenolics function as ROS scavengers, osmolyte, proteins and membrane stabilizer, and buffer of cellular redox reactions [5, 6]. Proline also helps in the maintenance of cellular-acidosis, protein-compatible-hydrotrope and balancing the ratio of $NADP^+$/NADPH essential for cellular metabolism. Brakedown of proline after stress, generates huge amount of reducing agents that are used for ATP synthesis in mitochondrial oxidative phosphorylation. ATPs are then used in stress retrieval and in the refurbishment of already done damages [18]. Phenolics are known to function as antioxidants, herbivores restrictors, and has the ability to attract plant pollinating insects [19]. Besides, plant hormones, such as abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) have a role in diverse ecological stresses as signaling chemicals [20-25]. Among these phytohormones, ABA help in opening and closing of stomata during drought and thermal stresses [26]. ABA also has a role in seed germination, maturation and senescence of vascular plants, including *Arabidopsis thaliana* [27].

Endophytic fungi, such as *Aspergillus flavus* [5], *Aspergillus japonicas* [6], *Penicillium roqueforti* [28], *Trichoderma reesei* [29] are asymptomatic beneficial microbes that reside inside the plant tissues on the permanent basis. Endophytes and rhizospheric microbes are known to have a role in the restoration of the host growth under various abiotic and biotic stresses. The endophytes provide resistance against stresses, accelerate mineral absorption and improve biomass of their host plant [30, 31]. Without endophytic fungi and rhizospheric microbes, plants are more susceptible to ecological constrains, like high temperatures, drought and salinity [5, 32-35]. *Aspergillus sydowii* was found to have the capability to tolerate heavy metals (HMs) toxicity by modifying the activities of CAT, GR, SOD and the contents of malondialdehyde, proline and thiol [36]. The present work was performed in an attempt to investigate the role of *P. glabrum* as a stress adaptive tool in *G. max* and *H. annuus* during heat stress.

Materials and Methods

Experiments were conducted at the Department of Botany, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan. *Oryza sativa* L. seeds, Fakhr-e-Malakand (commercial variety) were attained from the Agriculture Research Station, Mingora, Swat, Pakistan. *O. sativa* seeds were grown in pots containing 30 ml of water-agar medium (0.8% w/v). The pots were placed in a growth-chamber set at day light regime of 14 h, 28ºC±0.3 (day); and 10 h, 25ºC±0.3 (night) and 70% relative humidity. Growth promoting or inhibiting potential of the culture filtrate of fungal isolates was screened on *O. sativa* seedlings because of their quick and easy reply to growth promoting phytohormones, like indole acetic acid (IAA) and gibberellins (GAs). The best growth promoting endophytes were inoculated to oil producing *G. max* and *H. annuus* crops, exposed to heat stress. *G. max* (Swat 84) and *H. annuus* (ICI Hyson 33 Australia) varieties were grown in autoclaved sand for two weeks in growth chambers. The conditions of one of the growth chambers (control experiment) was set at 28ºC±0.3, 14 h (day), and 25ºC±0.3, 10 h (night). Whereas, the conditions of the other growth chamber set for heat stress experiment was 14 h, 40ºC±0.3 (day), 10 h, 35ºC±0.3 (night). The relative humidity in both cases was kept at 70%. All experiments were performed in triplicates.

Isolation of Fungal Endophytes

Methodology of Khan, et al. [37] was applied for the isolation of fungal endophytes from *Dryopteris blanfordii*. A wild medicinal plant *Dryopteris*, was collected from dry rocky sun-side mountain of District Swat, Tehsil Kabal (DD COORDINATES: 34.7833302 72.2833322; DMS COORDINATES: 34°46'59.99" N 72°16'60.00" E; GEOHASH COORDINATES: tw5muu55edxn8; UTM COORDINATES: 43S 251415.10226142 3852379.1095829), Khyber Pakhtunkhwa, Pakistan in the month of July. The plants were carried to plant microbes' interaction (PMI) laboratory. *Dryopteris* was selected for the isolation of endophytes because many of the medicinal plant species, we have explored previously served as a pool of potential endophytes. Initially fungal isolates were grown in Hagem media, which was then purified using a PDA (potato dextrose agar) media. For the collection of secondary metabolites and biomass, the purified isolates were inoculated to Czapek media. The Czapek media were incubated for 7 days in a shaking incubator operated at 120 rpm.

Initial Screening of Isolated Fungi on *O. sativa* Seedlings

Initially, the isolated fungal strains were screened on *O. sativa* seedlings for their growth promoting potential at two leaves phase. Culture filtrate (100 µl) f the fungal strains was applied to the tip of the *O. sativa* seedlings, grown in 0.8% water-agar medium. The plants were transferred to the growth chambers operated under conditions as described earlier and incubated for one week. Growth attributes, including total chlorophyll content, root and shoot length, fresh and dry weight of root and shoot, were measured and compared with distilled water and Czapek media as control [38].

Molecular Identification of Fungal Isolate

Identification of fungal isolate was carried out using molecular tools by the well described procedure of Khan et al. [39, 40]. Amplification of 18S rDNA was performed with ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) forward and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) reverse primers [41]. Approximation of sequence homology was carried out using BLASTn1 software for the attained sequence. For phylogenetic analysis, MEGA 7 software was used and trees were constructed with Neighbor Joining.

Inoculation of *P. glabrum* to *G. max* and *H. annuus*

Biomass of *P. glabrum* was applied to the pots containing autoclaved sand and seeds of *G. max* and *H. annuus* (6 seeds/pot). The pots were then transferred to the growth chamber operated under condition as described earlier. Minerals were applied in the form of Hoagland solution. Growth attributes of endophyte-inoculated and non-inocu,ated *G. max* and *H. annuus* seedlings were noted and compared after 2 weeks of fungal inoculation [42].

Determination of Antioxidants

Fresh leaves (10 gram) of *G. max* or *H. annuus* were soaked in Sodium-phosphate buffer (50 mm, pH 7) containing 1% polyvinyl-pyrolidine (w/v). The mixture was spun at 4ºC and 15,000 rpm for twenty minutes. Supernatant was used to analyze the enzymatic activity. Catalase (EC 1.11.1.6) activity was determined using Luck [43] procedure with some modifications. Extinction co-efficient (36×10^3 mM^{-1} cm^{-1}) was applied to measure CAT activity and was shown as EU mg^{-1} protein. Kar and Mishra [44] method was applied for POD, EC 1.11.1.7) activity. The mixture contained H_2O_2 (50 µM), H_3PO_4 buffer (125 µM pH 6.8), pyrogallol (50 µM) and 20X diluted enzyme extract (1 ml) in a 5 ml total volume. Quantity of the developed purpurogallin was monitored at 420 nm. POD concentration was calculated as EU mg^{-1} protein. Beyer Jr and Fridovich [45] protocol was adopted for the investigation of SOD (EC 1.15.1.1) activity. SOD activity was monitored by noting a decrease in the absorbance of nitro-blue tetrazolium (NBT). SOD concentration was determined as EU mg^{-1} protein. Carlberg and Mannervik [46] method was applied for the activity of GR (GR, EC 1.6.4.2). GR was measured by the reduction in OD at 340 nm for two minutes. GR concentration was measured by applying an extinction co-efficient of 0.12 mM NADPH, which was 6.2 mM^{-1} cm^{-1}, and was shown as EU mg^{-1} protein.

Determination of ABA

ABA concentration was determined in the leaves of *G. max* and *H. annuus* using optimized protocol of Yoon, et al. [18]. Fresh leaves (0.5 g) of *G. max* and *H. annuus* were crushed using liquid N_2 in a pestle and mortar. Glacial acetic acid (28.5 ml) and isopropanol (1.5 ml) were added to the sample. The mixture was then filtered and dehydrated through rotary evaporator. Diazo-methane was added to this mixture and examined via GC–MS SIM (6890N network GC system furnished with 5973 network mass-selective-detector, Agilent Technologies, Palo Alto, CA, USA). The Lab-Base (Thermo Quset, Manchester, UK) data system software was used to detect retorts to ions with m/z values of 162 and 190 for Me-ABA and 166 and 194 for Me-[2H6]-ABA. ABA ([2H6]-ABA) (Sigma Aldrich) was used as an internal standard.

Determination of Phenolics and Proline

Total proline contents were analyzed via methodology of Bates, et al. [47]. Various concentrations

of proline (Sigma Aldrich; 2, 4, 6, 8, and 10 µg/ml) were used to plot a standard curve. Optical density was noted at 520 nm.

Cai, et al. [48] methodology of Folin-Ciocalteu colorimetric was followed to examine phenolics in *G. max* and *H. annuus*. Sample (0.2 ml) and 0.5 N Folin-Ciocalteu reagent were mixed and kept for 4 min at 25ºC. Sodium carbonate (75 g/l) was used for the neutralization of mixture and the mixture was heated at 100ºC for 1 minute. This mixture was then kept in the dark for 2 hours. Optical density was taken at 650 nm. Various concentrations of gallic acid were prepared to construct a standard curve (100, 200, 300, 500 and 600, 700 and 900 mg/ml).

Determination of Total Lipids, Proteins and Sugars

Proteins were determined in *H. annuus* and *G. max* seedlings using methodology of Lowry, et al. [49]. Homogenate of filtered leaf sample (0.1 ml) was mixed with 0.1 ml of 2M NaOH. The mixture was heated at 100ºC to hydrolyse. The resulted hydrolysate was cooled and mixed with 1 ml of complex-forming solution [complex-forming solution = 2% (w/v) Na_2CO_3 in distilled H_2O, 1% (w/v) $CuSO_4.5\ H_2O$ in distilled H_2O, 2% (w/v) sodium potassium tartrate in distilled H_2O] and let the mixture stand for 10 minutes at room temperature. About, 1 ml of Folin reagent was added and the mixture was vortexed. The mixture was allowed to stand for 30 minutes at room temperature. The absorbance was finally read at 650 nm using spectrophotometer. Different concentrations of bovine serum albumin (BSA) (Sigma Aldrich; 20, 40, 60, 80, and 100 µg/ml) was applied to draw a standard curve. Van Handel [50] methodology was followed for the analysis of total lipids. Crushed leaf sample was centrifuged and about 1 ml of the supernatant was taken and transferred to the reaction tube containing 1 ml chloroform/methanol (1:2 v/v) solution. The mixture was heated in a water bath at 90ºC until complete evaporation. Concentrated sulfuric acid (2 ml) was added to the precipitate formed and mixed with the help of vortex for 2 minutes and then heated on a water bath at 90ºC. The mixture was cooled and added 5 ml vanillin-phosphoric acid reagent. The tube containing mixture was allowed to stand for 30 minutes at room temperature for colour development. Finally the tube was mixed and the absorbance was measured at 525 nm. Various concentrations of pure canola oil (10, 40, 70, 100, 130, and 160 µg/ml) was used to draw a standard curve. Mohammadkhani and Heidari [41] protocol was applied to determine soluble sugar in *H. annuus* and *G. max* leaves. Homogenate of filtered leaf sample (1 ml) was mixed with 10 µl of 5% phenol and 1 ml of 98% sulfuric acid. The mixture was vortexed and allowed to stand for 1h. The absorbance was finally measured at 485 nm by using a spectrophotometer. Different concentrations of glucose (Sigma Aldrich; 20, 40, 60, 80, and 100 µg/ml) were taken to plot a standard curve.

Statistical Analysis

Experiments were accomplished in triplicates. ANOVA was applied to analyze the data. Mean values were equated by DMRT (Duncan Multiple Range Test) at $p<0.05$, via SPSS-20 (SPSS Inch., Chicago, IL, USA).

Results and Discussion

Isolation Fungal Endophytes and Their Plant Growth Promoting Activity

Nine different fungal isolates were collected from *Dryopteris blanfordii*. The growth promotion or inhibition potential of the endophytes was screened on *O. sativa* plantlets. The *P. glabrum*-treated *O. sativa* seedlings had more chlorophyll content (7%), shoot (37%) and root length (5%). Similarly, higher weights were recorded for the fresh (36%) and dry shoot (70%), and fresh (9%) and dry roots (5%) in comparison to the Czapek-treated control seedlings (Table 1).

Table 1. Effect of *P. glabrum* filtrate on the growth of *O. sativa* seedlings.

Growth attributes	Control (DW)	Control (Czk)	*P. glabrum*
Shoot Length (cm)	10.4±0.7[a]	10.6±1.4[a]	14.5±0.7[b]
Root Length (cm)	4.9±1.4[a]	6.3±0.5[a]	6±1.0[a]
Fresh Weight Shoot (g)	0.03±.05[a]	0.0317±0.05[a]	0.043±0.02[b]
Fresh Weight Root (g)	0.08±0.01[a]	0.095±0.01[a]	0.1037±0.08[b]
Dry Weight Shoot (g)	0.0047±0.0005[a]	0.0043±0.0005[a]	0.0073±0.0005[b]
Dry Weight Root (g)	0.014±0.001[a]	0.015±0.002[a]	0.016±0.001[a]
Chlorophyll content (SPAD)	18.9±2.1[a]	21.4±0.6[ab]	22.9±0.6[b]

Czk = Czapek medium, DW = Distilled water. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

DryR-30 isolate was the best growth promoter of *O. sativa* seedlings, which was selected for further analysis.

Identification of Fungal Isolate DryR-30

Internal Transcribed Spacer (ITS) region of the nucleotide sequence of DryR-30 isolate was equated with allied strains through the BLAST search program (Basic Local Alignment Search Tool, 2012). 18S rDNA sequence revealed maximum resemblance (54%) with *P. glabrum*. The phylogenetic harmony tree was prepared from 1 cloned and 8 references via NJ (Neighbor Joining) technique using MEGA-7.0 software (Fig. 1). Results of sequence homology and phylogenetic examination proposed DryR-30 as *P. glabrum*.

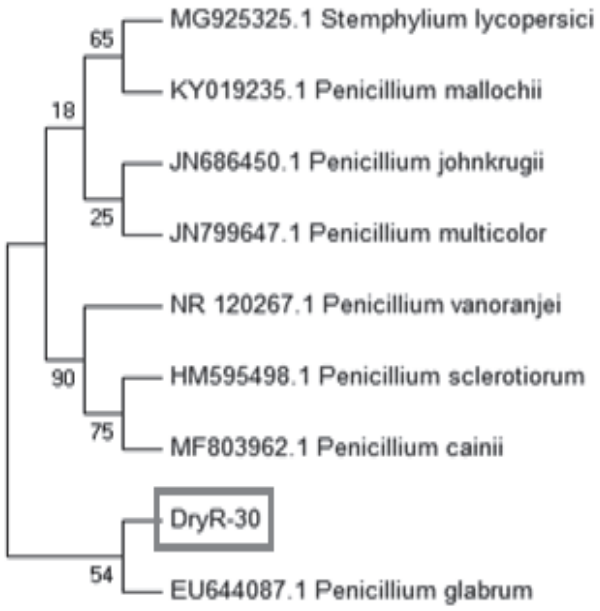

Fig. 1. Phylogenetic consensus tree construction (using 9 taxa, 8 reference and 1 clone) for the identification of fungal isolate DryR-30 using neighbor joining (NJ) method. 54% bootstrap value confirmed isolate DRYR-30 as *Penicillium glabrum.*

Growth Attributes of *G. max* and *H. annuus* Allied to *P. glabrum*

Plant growth characteristics, such as total chlorophyll content, root-shoot length, and fresh and dry weight of root and shoot of experimental plants (*P. glabrum*-co-cultured) were measured and compared with control plants. All the plants were grown for two weeks, at 25ºC and 40ºC. A significant increase in all growth attributes was found in *G. max* and *H. annuus* plantlets allied with *P. glabrum* as compared to control plantlets. Endophyte-associated *G. max* has enhanced chlorophyll (4%), shoot length (34.6%), root length (40%), shoot (28.5%) and root fresh weights (6%), and shoot (2%) and root dry weights (24.5%), when exposed to thermal stress (Table 2). Similarly, the increase was also recorded in the chlorophyll content (3%), shoot (6%) and root length (20%), fresh shoot (18%) and fresh root weight (53%), dry shoot 35%) and dry root weight (33%) of *P. glabrum*-inoculated *H. annuus* at heat stress as compared to control plants (Table 3). We observed a high content of chlorophyll in *P. glabrum*-inoculated *G. max* and *H. annuus* as compared to control seedlings. which might be due to enhancement in the uptake of Mg element by *P. glabrum*. In a similar experiment a high rate of photosynthesis was noted in *Capsicum annuum* colonized by an endophytic fungi *Penicillium resedanum* [51] and *Chaetomium globosum* [52] under drought stress.

Intercession of Physiological Characteristics

Higher concentration of flavonoids and phenolics, while reduced levels of proline contents were recorded in *G. max* and *H. annuus* allied with *P. glabrum* under heat stress as compared to control plants (Fig. 2). The maximum increase (15.7%) in phenolic contents was noted in the seedlings of *H. annuus*, whereas the fungal strain was less responsive in *G. max* (5.6%) seedlings

Table 2. Effect of *P. glabrum* on the growth features of *G. max.*

Growth attributes/ temperature stress	25ºC		40ºC	
	Control	*P. glabrum*	Control	*P. glabrum*
Shoot Length (cm)	38.7±1.7^{b}	45.7±2.1^{c}	26±0.9^{a}	35±1.1^{b}
Root Length (cm)	16±3.1^{a}	24±1.6^{b}	10±.5^{a}	14±1.8^{a}
Fresh Weight Shoot (g)	1.14±.02^{ab}	1.42±0.2^{b}	0.82±0.3^{a}	1.06±.02^{a}
Fresh Weight Root (g)	0.18±0.01^{ab}	0.32±0.08^{b}	0.13±0.1^{a}	0.12±0.03^{ab}
Dry Weight Shoot (g)	0.13±0.01^{c}	0.12±0.09^{c}	0.07±0.003^{a}	0.09±0.001^{b}
Dry Weight Root (g)	0.065±0.005^{b}	0.075±0.002^{c}	0.010±0.001^{a}	0.013±0.003^{a}
Total Chlorophyll (SPAD)	29±1.9^{a}	43±1.1^{b}	28.9±0.5^{a}	30±0.2^{a}

Effect of *P. glabrum* on *G. max* seedlings, isolated from *D. blanfordii*. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

Table 3. Effect of *P. glabrum* on the growth features of *H. annuus.*

Growth attributes/ temperature stress	25°C		40°C	
	Control	*P. glabrum*	Control	*P. glabrum*
Total Chlorophyll (SPAD)	39±1.2^{a}	42.6±2.5^{a}	37.4±0.6^{a}	38.4±1.9^{a}
Shoot Length (cm)	24.5±0.8^{b}	28.9±0.8^{c}	20±0.4^{a}	23±0.8^{b}
Root Length (cm)	9.0±0.5^{b}	11.9±0.5^{c}	5.0±0.2^{a}	6.0±0.5^{a}
Fresh Weight Shoot (g)	1.22±0.09^{ab}	1.421±0.05^{b}	0.928±0.2^{a}	1.095±0.04^{ab}
Fresh Weight Root (g)	0.131±0.02^{ab}	0.188±0.04^{b}	0.099±0.006^{a}	0.152±0.06^{ab}
Dry Weight Shoot (g)	0.088±0.003^{b}	0.147±0.001^{c}	0.063±0.001^{a}	0.085±0.009^{b}
Dry Weight Root (g)	0.024±0.001^{a}	0.103±0.006^{c}	0.0345±0.004^{a}	0.046±0.002^{b}

Effect of *P. glabrum* on *H. annuus* seedlings, isolated from *D. blanfordii*. Data are means of 3 replicates with standard error. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

in terms of rise in phenolics concentrations as equated to fungal-free seedlings. In contrast proline content was significantly reduced (19.7%) in the seedlings of *G. max* as compared to *H. annuus* (16.8%). Among the defensive compounds accumulated in higher plants, phenolics are known to have vital role against biotic and abiotic stresses [6]. Detection of high phenolic contents in endophyte-allied *G. max* and *H. annuus* plants under

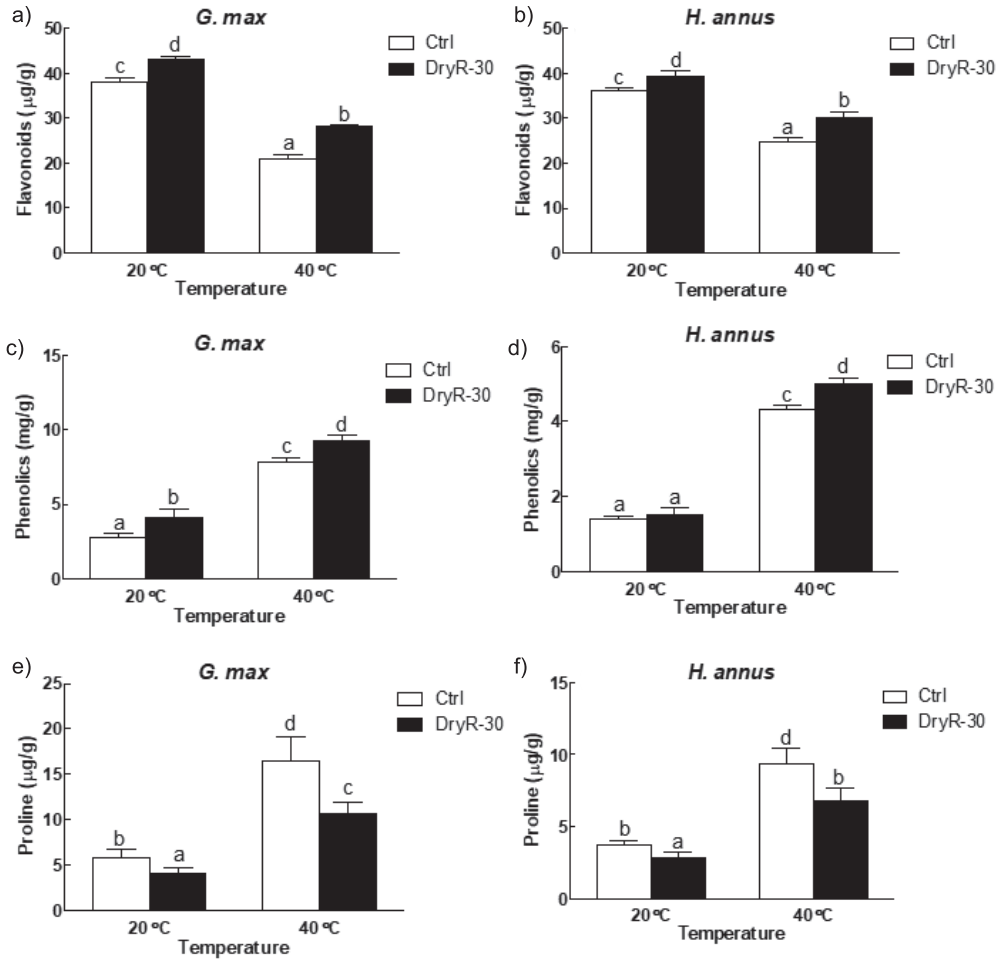

Fig. 2. Flavonids a), phenolics b) and proline c) in *G. max* and *H. annuus* inoculated with and without *P. glabrum*. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

thermal stress helped in mitigation of stress in plants [6]. Our results are similar to the work of Abd_Allah, et al. [53], who suggested that endophytes enhance phenolics content of their host plant under abiotic stress. Proline is known to function as organic osmolyte that accrue in various plants in response to different environmental stresses, including high temperature. A rise in proline was also noted in *P. glabrum* associated *G. max* and *H. annuus*. Such rise in proline content has an encouraging affect on plant endogenous enzymes, membrane integrity, free radicals scavenging, cellular redox potential and osmotic modification in plants during stress [54]. Moreover, proline has a role in relieving cytoplasmic acidosis, protein compatible hydrotrope and keeping suitable ratio of NADP+/NADPH [55].

A decline was noted in the concentration of ABA, in the leaves of endophyte-inoculated *G. max* and *H. annuus* seedlings at 25ºC and 40ºC. *G. max* seedlings co-cultured with *P. glabrum* has 1% lesser ABA as equated to control seedlings at 25ºC, while at 40ºC endophyte-inoculated *G. max* had 47% lesser ABA. Likewise, *P. glabrum*-inoculated *H. annuus* has 2% lesser ABA content at 25ºC, while 30% lesser ABA at 40ºC as compared to the endophyte-free plants (Fig. 3 a-b). High level of H_2O_2 and lipid peroxidation were recorded in endophyte-free *G. max* and *H. annuus* seedlings, subjected to heat stress (i.e. 40ºC). Elevation in H_2O_2 content is known to have a negative impact on membrane integrity. Under normal temperature (25ºC), a reduced H_2O_2 levels were found in *P. glabrum*-aligned *G. max* (41%) and *H. annuus* (48%), respectively. While, at thermal stress (40ºC) this decrease was 65% and 31%, respectively (Fig. 3 c,d). Quantification of lipids peroxidation was calculated in relation to MDA synthesis. A significant decrease was found in the amount of MDA in *P. glabrum*-aligned *G. max* and *H. annuus*. At 25ºC the decrease was 40% and 43% in the *G. max* and *H. annuus* seedlings, respectively, while at 40ºC the decrease was more in *H. annuus* (37%) than in *G. max* (77%) seedlings (Fig. 3 e-f). Abscisic acid accumulates in higher plants exposed to various environmental stresses, including high temperature. Elevated content of ABA was observed in a similar study in *O. sativa* under thermal stress [56]. Thermal stress causes up-regulation of ABA biosynthesis genes, while down-regulates those genes

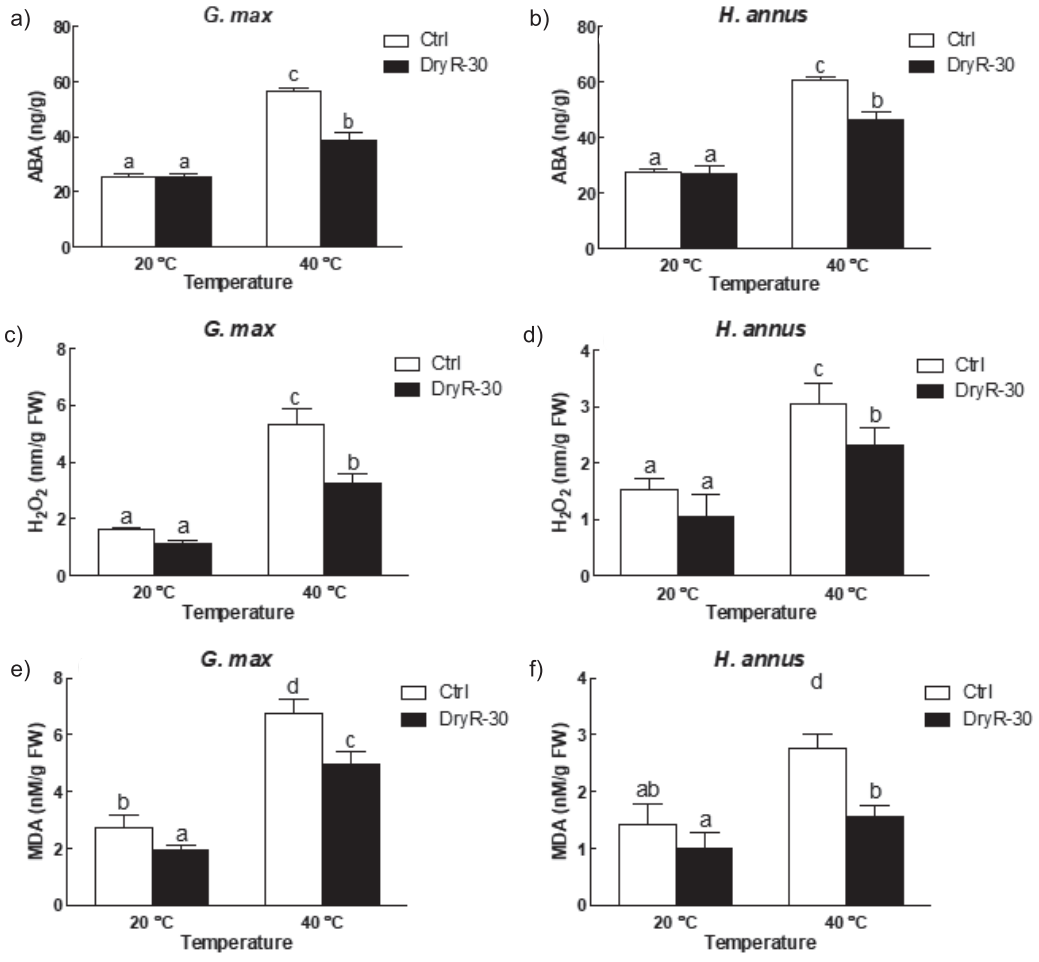

Fig. 3. ABA a) Hydrogen peroxide (H_2O_2) b) and Lipid peroxidation (MDA) c) in *G. max* and *H. annuus* inoculated with and without *P. glabrum*. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

Table 4. Effect of *P. glabrum* on the activity of antioxidant enzymes in *G. max.*

Enzyme/temperature stress	25ºC		40ºC	
	Control	*P. glabrum*	Control	*P. glabrum*
AAO (Unit/g/30 sec.)	1.56±0.08[a]	1.78±0.03[a]	1.80±0.12[b]	2.09±0.12[c]
CAT (Unit/g/30 sec.)	0.42±0.03[a]	0.60±0.02[b]	0.93±0.04[c]	1.14±0.08[d]
POD (U/mg protein)	1.16±0.08[a]	1.39±0.18[b]	4.61±0.57[c]	5.73±0. 21[d]
SOD (U/mg protein)	20±1.89[a]	23±0.70[a]	43±1.45[b]	57±1.04[c]
GR (U/mg protein)	1.37±0.19[a]	1.44±0.19[a]	1.63±0.03[b]	2.01±0.13[c]

Effect of *P. glabrum* on *H. annuus* seedlings, isolated from *D. blanfordii*. AAO = ascorbic acid oxidase; CAT = catalase; POD = peroxidase, SOD = superoxide dismutase; GR = glutathione reductase. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

which are responsible for ABA catabolism [57]. In present work, elevated contents of ABA was noted in sunflower and soybean inoculated with *P. glabrum* subjected to high temperature stress as compared to non-inoculated seedlings. This reduction in ABA content in experimental plants might be due to down-regulation of genes responsible for ABA synthesis or acceleration of ABA degradation. Low amount of ABA in fungal endophyte associated *G. max* and *H. annuus* confirmed the findings of Hamayun, et al. [21]. Low amount of ABA in endophyte-associated plants may be due to the participation of GAs, as exogenous application of GAs enhance stress tolerance in *G. max* escorted by reduced amount of ABA. Plants initiate some intricate biosynthetic responses, like antioxidant systems, adjustments in membrane lipids, osmotic potential, and synthesis of heat-shock-proteins, under heat stress [53]. In the present study, we detected that *P. glabrum*-associated *G. max* and *H. annuus* seedlings are more resistant to thermal stress than non-allied seedlings. It is known that plant′s biochemical, physiological and transcriptional mechanisms are severely affected by thermal stress. Oxidative stress results in the accumulation of high concentration of MDA in control plants due to lipids peroxidation. In our study, both *G. max* and *H. annuus* control seedlings exposed to heat stress, have elevated levels of MDA as compared to *P. glabrum*-associated plants. The rise in MDA concentration is injurious to plants that cause membrane damaging, while endophytes are known to reduce the impacts of abiotic stresses by reducing the level of MDA in their host plants [5]. In one such type of work, 27.5% decrease in the levels of MDA was found in Chickpea plants inoculated with *Bacillus subtilis* BERA 71 facing 200 mM NaCl salt stress [58]. Our study confirmed the work of Abd_Allah, et al. [53] on chickpea that endophytes ameliorate abiotic stresses by reducing the levels of MDA and H_2O_2. As stated earlier that thermal stress boosts up H_2O_2 in plants, which might affect membrane structural integrity and directs lipids peroxidation, hence more MDA synthesis, which is noted in control *G. max* and *H. annuus* seedlings [5, 6, 14].

A considerable rise was seen in antioxidants activities, including AAO, CAT, POD, SOD and GR in endophyte-aligned *G. max* and *H. annuus* seedlings at 40ºC as equated to non-aligned *P. glabrum* seedlings. *P. glabrum*-aligned *G. max* seedlings have enhanced concentrations of CAT (20%), POD (49%), AAO (13%), SOD (24%) and GR (28%) (Table 4). Similarly, enhanced concentration of CAT (27%) POD (28%), AAO (13.6%), SOD (21%) and GR (40%) was also

Table 5. Effect of *P. glabrum* on the activity of antioxidant enzymes in *H. annuus.*

Enzyme/temperature stress	25ºC		40ºC	
	Control	*P. glabrum*	Control	*P. glabrum*
AAO (Unit/g/30 sec.)	0.64± 0.06[a]	0.87±0.06[b]	2.26±0.36[c]	2.58±0.28[c]
CAT (Unit/g/30 sec.)	0.23±0.03[a]	0.37±0.02[b]	0.55±0.04[c]	0.75±0.03[d]
POD (U/mg protein)	0.98±0.07[a]	1.23±0.02[b]	3.54±0.57[c]	4.15±0.08[d]
SOD (U/mg protein)	14±0.91[a]	20±0.98[b]	27±1.13[c]	36±1.78[d]
GR (U/mg protein)	0.60± 0.06[a]	0.57±0.12[a]	1.95±0.15[b]	2.56±0.07[c]

Effect of *P. glabrum* on *H. annuus* seedlings, isolated from *D. blanfordii*. AAO = ascorbic acid oxidase; CAT = catalase; POD = peroxidase, SOD = superoxide dismutase; GR = glutathione reductase. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

recorded in *P. glabrum*-aligned *H. annuus* as compared to endophyte-free seedlings (Table 5). The ameliorative role of *P. glabrum* for *G. max* and *H. annuus* against heat stress might be due to the up-regulation of nutrients uptake and antioxidant system, and decline of ROS [59]. The enzymatic antioxidant system of plants, include CAT, POD, SOD, AAO and GR. The up-regulation of these antioxidants protect plant´s membrane from the hazardous effects of free radicals generated under various stresses [53]. SOD is an important antioxidant enzyme that functions as scavenging of H_2O_2 and superoxide radicals for the synthesis of hydroxyl (OH-) radicals and decline of Haber-Weiss reaction. It is also known that up-regulation of CAT, POD, SOD and GR reduced membrane dysfunction in chickpea. Stress toxicity is reduced by high content of POD that might be due to accelerating lignins biosynthesis and other protective compounds [60]. Enhanced activity of CAT boost up immunity against abiotic stress in *Brassica juncea* [61]. CAT function as eliminator of H_2O_2 to protect membranes and organelles damage because H_2O_2 is known as signaling molecule, which quickly diffuses through membrane [62]. AAO and GR are important compounds of ROS degrading pathway that comprises the ascorbate-glutathione cycle [56]. A sequence of oxidation-reduction reactions arises in the ascorbate-glutathione cycle leading to eliminate H_2O_2 in cytosol and chloroplasts to reduce the toxicity of oxidative-stress on plants under abiotic stress. Rise in the activity of AAO and GR in heat affected seedlings might be owing to the safeguarding of photosynthetic electron transport chain (PETC) by keeping balance of NADP+/NADPH ratio for unceasing stream of electrons to O_2, leading in abridged superoxide radicals' creation [56].

Lipids, proteins and sugars of *G. max* and *H. annuus* were determined via spectrophotometer. Endophyte-inoculated *G. max* has 56% and *H. annuus* has 75% more protein at 40 °C, while at 25ºC *G. max* had 44% and *H. annuus* had 39% more proteins as compared to the endophyte-free plants (Fig. 4a-b). At 40ºC, *P. glabrum*-aligned *G. max* had 32% and *H. annuus* had 54% more soluble sugars, while at 25ºC *G. max* had 19% and *H. annuus* had 38% more soluble sugars as equated to *P. glabrum*-free seedlings (Fig. 4c-d). Moreover, an increase of 27% and 26% in the total lipid contents

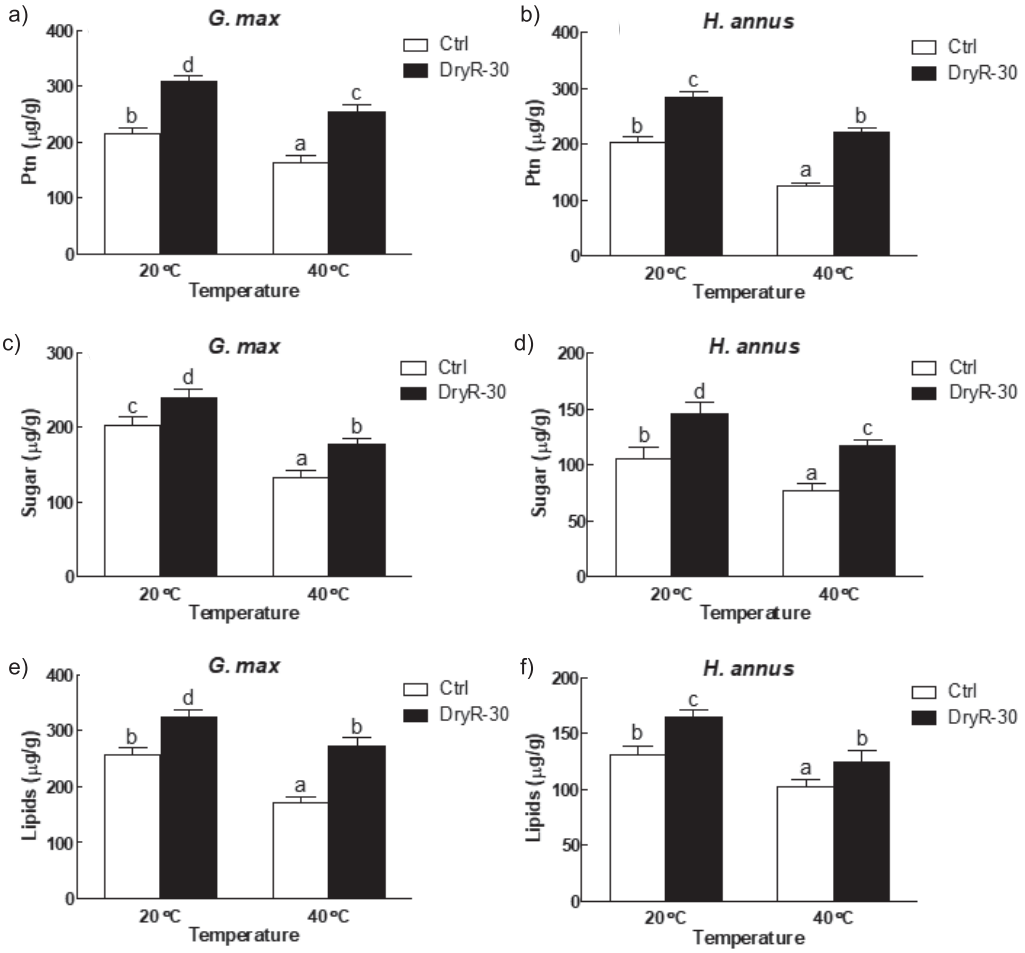

Fig. 4. Analysis of total proteins s), soluble sugars b) and lipids c) of *G. max* and *H. annuus* inoculated with *P. glabrum*. Data are means of 3 replicates with standard error. Different letters are significantly different ($p<0.05$) as estimated by Duncan's Multiple Range Test (DMRT).

of *P. glabrum*-inoculated *G. max* and *H. annuus* were recorded at 25ºC as compared to the non-inoculated plants (Fig. 4). At 40ºC, *P. glabrum*-associated *G. max* (60%) and *H. annuus* (21%) had more lipids as compared to control seedlings (Fig. 4 e-f). Improvement in the total nutritional value (lipids, proteins and sugars) of *P. glabrum*-associated *G. max* and *H. annuus* was found under thermal stress, signifying the beneficial role of endophytic fungi for crops and hence their use as bio-fertilizers.

Conclusion

It is well known that thermal stress is one of the major restraints of food crops, including *G. max* and *H. annuus*. So, this work signifies thermo-tolerant potential of *P. glabrum*. *P. glabrum* not only enhanced growth in *G. max* and *H. annuus*, but also facilitated it to resist heat stress by boosting ROS scavenging antioxidant system of host plants. Furthermore, *P. glabrum* also tempered endogenous level of ABA in *G. max* and *H. annuus* as well as boosted their nutritional value. The present study suggests the use of *P. glabrum* as heat stress alleviating bio-agent and bio-fertilizer in the future for sustainable agriculture.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B04035601).

Conflict of Interest

The authors declare no conflict of interest.

References

1. PORCEL R., AROCA R., RUIZ-LOZANO J.M. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron. Sustain. Dev. **32** (1), 181, **2012**.
2. FAHAD S., BAJWA A. A., NAZIR U., ANJUM S. A., FAROOQ A., ZOHAIB A., SADIA S., NASIM W., ADKINS S., SAUD S. Crop production under drought and heat stress: plant responses and management options. Front. Plant Sci. **8**, 1147, **2017**.
3. FAHAD S., HUSSAIN S., SAUD S., HASSAN S., CHAUHAN B.S., KHAN F., IHSAN M. Z., ULLAH A., WU C., BAJWA A.A. Responses of rapid viscoanalyzer profile and other rice grain qualities to exogenously applied plant growth regulators under high day and high night temperatures. PLoS One. **11** (7), e0159590, **2016**.
4. FAHAD S., IHSAN M. Z., KHALIQ A., DAUR I., SAUD S., ALZAMANAN S., NASIM W., ABDULLAH M., KHAN I.A., WU C. Consequences of high temperature under changing climate optima for rice pollen characteristics-concepts and perspectives. Archives of Agronomy and Soil Science. **64** (11), 1473, **2018**.
5. ISMAIL, HAMAYUN M., HUSSAIN A., AFZAL KHAN S., IQBAL A., LEE I.-J. *Aspergillus flavus* promoted the growth of soybean and sunflower seedlings at elevated temperature. BioMed Research International. **2019**, 1, **2019**.
6. ISMAIL, HAMAYUN M., HUSSAIN A., IQBAL A., KHAN S.A., LEE I.-J. Endophytic fungus *Aspergillus japonicus* mediates host plant growth under normal and heat stress conditions. BioMed Research International. **2018**, 1, **2018**.
7. ALI S., KHAN S.A., HAMAYUN M., IQBAL A., KHAN A.L., HUSSAIN A., SHAH M. Endophytic fungi from Caralluma acutangula can secrete plant growth promoting enzymes. Fresenius Environmental Bulletin. **28**, 2688, **2019**.
8. ISMAIL, ANWAR H., MEHMOOD A., QADIR M., HUSNA, AMJAD I., HAMAYUN M., KHAN N. Thermal stress alleviating potential of endophytic fungus Rhizopus Oryzae Inoculated To Sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.). Pak. J. Bot. **52**, 1, **2020**.
9. JAMIL M., ALI A., GHAFOOR A., GUL A., AKBAR K.F., BASHIR H., IJAZ A., HUSSAIN R., MUNEEB A., NAVEED N.H. Yield reduction analysis of bread wheat under heat stress at two different environments in Pakistan. Fresenius Environmental Bulletin. 4602, **2017**.
10. ABDELAAL A., OMARA I., HAFEZ M., SAMAR M., EL SABAGH A. Anatomical, biochemical and physiological changes in some Egyptian wheat cultivars inoculated with Puccinia graminis f. sp. tritici. Fresenius Environmental Bulletin. **27** (1), 296, **2018**.
11. ISMAIL, ANWAR H., MEHMOOD A., QADIR M., HUSNA, IQBAL A., HAMAYUN M., KHAN N. Thermal stress alleviating potential of endophytic fungus *Rhizopus oryzae* inoculated to sunflower (*Helianthus annuus* L.) And soybean (*Glycine max* L.). Pakistan Journal of Botany. **52**, 1, **2020**.
12. ISMAIL, HAMAYUN M., ANWAR H., SUMERA AFZAL K., AMJAD I., IN-JUNG L. An endophytic fungus *Aspergillus violaceofuscus* can be used as heat stress adaptive tool for *Glycine max* L. and *Helianthus annuus* L. Journal of Applied Botany and Food Quality. **93** 112, **2020**.
13. ISMAIL, HAMAYUN M., HUSSAIN A., AFZAL KHAN S., IQBAL A., LEE I.-J. *Aspergillus niger* boosted heat stress tolerance in sunflower and soybean via regulating their metabolic and antioxidant system. Journal of Plant Interactions. **15**, 223, **2020**.
14. ISMAIL, MUHAMMAD H., ANWAR H., AMJAD I., SUMERA A.K., IN-JUNG L. *Aspergillus niger* boosted heat stress tolerance in sunflower and soybean via regulating their metabolic and antioxidant system. Journal of Plant Interactions. **15**, 223, **2020**.
15. ALQARAWI A.A., ABD ALLAH E., HASHEM A. Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. J. Plant Interact. **9** (1), 802, **2014**.
16. GHORBANPOUR M., HATAMI M., KHAVAZI K. Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress. Turk J Biol. **37** (3), 350, **2013**.
17. ABD-ALLAH E.F., HASHEM A., ALQARAWI A.A., BAHKALI A.H., ALWHIBI M.S. Enhancing growth

performance and systemic acquired resistance of medicinal plant *Sesbania sesban* (L.) Merr using arbuscular mycorrhizal fungi under salt stress. Saudi J. Biol. Sci. **22** (3), 274, **2015**.
18. YOON J. Y., HAMAYUN M., LEE S.-K., LEE I.-J. Methyl jasmonate alleviated salinity stress in soybean. Journal of Crop Science and Biotechnology. **12** (2), 63-68, **2009**.
19. KANG J., HWANG J.-U., LEE M., KIM Y.-Y., ASSMANN S. M., MARTINOIA E., LEE Y. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc. Natl. Acad. Sci. **107** (5), 2355, **2010**.
20. HAMAYUN M., HUSSAIN A., KHAN S.A., IRSHAD M., KHAN A.L., WAQAS M., SHAHZAD R., IQBAL A., ULLAH N., REHMAN G. Kinetin modulates physio-hormonal attributes and isoflavone contents of Soybean grown under salinity stress. Front. Plant Sci. **6**, 377, **2015**.
21. HAMAYUN M., HUSSAIN A., KHAN S.A., KIM H.-Y., KHAN A.L., WAQAS M., IRSHAD M., IQBAL A., REHMAN G., JAN S. Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Frontiers in Microbiology. **8**, 686, **2017**.
22. KHAN M.A., HAMAYUN M., IQBAL A., KHAN S.A., HUSSAIN A., ASAF S., KHAN A. L., YUN B.-W., LEE I.-J. Gibberellin application ameliorates the adverse impact of short-term flooding on *Glycine max* L. Biochem. J. **475** (18), 2893, **2018**.
23. BILAL L., ASAF S., HAMAYUN M., GUL H., IQBAL A., ULLAH I., LEE I.-J., HUSSAIN A. Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. Symbiosis. **76** (2), 117, **2018**.
24. JAN F. G., HAMAYUN M., HUSSAIN A., JAN G., IQBAL A., KHAN A., LEE I.-J. An endophytic isolate of the fungus *Yarrowia lipolytica* produces metabolites that ameliorate the negative impact of salt stress on the physiology of maize. BMC Microbiology. **19** (1), 1, **2019**.
25. GUL JAN F., HAMAYUN M., HUSSAIN A., IQBAL A., JAN G., KHAN S.A., KHAN H., LEE I.-J. A promising growth promoting *Meyerozyma caribbica* from *Solanum xanthocarpum* alleviated stress in maize plants. Bioscience Reports. **39**, 1, **2019**.
26. KUROMORI T., MIYAJI T., YABUUCHI H., SHIMIZU H., SUGIMOTO E., KAMIYA A., MORIYAMA Y., SHINOZAKI K. ABC transporter AtABCG25 is involved in abscisic acid transport and responses. Proc. Natl. Acad. Sci. **107** (5), 2361, **2010**.
27. MIERZIAK J., KOSTYN K., KULMA A. Flavonoids as important molecules of plant interactions with the environment. Molecules. **19** (10), 16240, **2014**.
28. IKRAM M., ALI N., JAN G., JAN F. G., RAHMAN I. U., IQBAL A., HAMAYUN M. IAA producing fungal endophyte *Penicillium roqueforti* Thom., enhances stress tolerance and nutrients uptake in wheat plants grown on heavy metal contaminated soils. PloS One. **13** (11), e0208150, **2018**.
29. MUHAMMAD I., NIAZ A., GUL J., AMJAD I., MUHAMMAD H., FARZANA G. J., ANWAR H., IN-JUNG L. *Trichoderma reesei* improved the nutrition status of wheat crop under salt stress. Journal of Plant Interactions. **14**, 590, **2019**.
30. MEHMOOD A., HUSSAIN A., IRSHAD M., HAMAYUN M., IQBAL A., KHAN N. In vitro production of IAA by endophytic fungus *Aspergillus awamori* and its growth promoting activities in *Zea mays*. Symbiosis. **77**, 225, **2019**.
31. MEHMOOD A., HUSSAIN A., IRSHAD M., HAMAYUN M., IQBAL A., RAHMAN H., TAWAB A., AHMAD A., AYAZ S. Cinnamic acid as an inhibitor of growth, flavonoids exudation and endophytic fungus colonization in maize root. Plant Physiol. Biochem. **135**, 61, **2019**.
32. KANG S.-M., HAMAYUN M., KHAN M. A., IQBAL A., LEE I.-J. *Bacillus subtilis* JW1 enhances plant growth and nutrient uptake of Chinese cabbage through gibberellins secretion. Journal of Applied Botany and Food Quality. **92**, 172, **2019**.
33. KHUSHDIL F., JAN F. G., JAN G., HAMAYUN M., IQBAL A., HUSSAIN A., BIBI N. Salt stress alleviation in *Pennisetum glaucum* through secondary metabolites modulation by *Aspergillus terreus*. Plant Physiology and Biochemistry. **144**, 127, **2019**.
34. QADIR M., HUSSAIN A., HAMAYUN M., SHAH M., IQBAL A., MURAD W. Phytohormones producing rhizobacterium alleviates chromium toxicity in *Helianthus annuus* L. by reducing chromate uptake and strengthening antioxidant system. Chemosphere. 127386, **2020**.
35. ADNAN M., FAHAD S., ZAMIN M., SHAH S., MIAN I.A., DANISH S., ZAFAR-UL-HYE M., BATTAGLIA M.L., NAZ R.M.M., SAEED B. Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. Plants. **9** (7), 900, **2020**.
36. MUKHERJEE A., DAS D., MONDAL S. K., BISWAS R., DAS T.K., BOUJEDAINI N., KHUDA-BUKHSH A.R. Tolerance of arsenate-induced stress in *Aspergillus niger*, a possible candidate for bioremediation. Ecotoxicol. Environ. Saf. **73** (2), 172, **2010**.
37. KHAN S.A., HAMAYUN M., YOON H., KIM H.-Y., SUH S.-J., HWANG S.-K., KIM J.-M., LEE I.-J., CHOO Y.-S., YOON U.-H. Plant growth promotion and *Penicillium citrinum*. BMC Microbiol. **8** (1), 231, **2008**.
38. KHAN S. A., HAMAYUN M., KIM H.-Y., YOON H.-J., SEO J.-C., CHOO Y.-S., LEE I.-J., RHEE I.-K., KIM J.-G. A new strain of *Arthrinium phaeospermum* isolated from *Carex kobomugi* Ohwi is capable of gibberellin production. Biotechnol. Lett. **31** (2), 283, **2009**.
39. ELAD Y., PERTOT I. Climate change impacts on plant pathogens and plant diseases. J. Crop Improv. **28** (1), 99, **2014**.
40. BORNMAN J.F., BARNES P.W., ROBINSON S.A., BALLARE C.L., FLINT S., CALDWELL M.M. Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems. Photochem. Photobiol. Sci. **14** (1), 88, **2015**.
41. MOHAMMADKHANI N., HEIDARI R. Drought-induced accumulation of soluble sugars and proline in two maize varieties. World Appl. Sci. J. **3** (3), 448, **2008**.
42. MISRA N., DWIVEDI U. Genotypic difference in salinity tolerance of green gram cultivars. Plant Sci. **166** (5), 1135, **2004**.
43. LUCK H. Methods in Enzymatic Analysis II (ed.) Bergmeyer. (Publ.), Academic Press, New York: **1974**.
44. KAR M., MISHRA D. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. **57** (2), 315, **1976**.
45. BEYER JR W.F., FRIDOVICH I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. **161** (2), 559, **1987**.
46. CARLBERG I., MANNERVIK B. [59] Glutathione reductase, Elsevier: 484, **1985**.

47. BATES L.S., WALDREN R.P., TEARE I. Rapid determination of free proline for water-stress studies. Plant and Soil. **39** (1), 205, **1973**.
48. CAI Y., LUO Q., SUN M., CORKE H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. **74** (17), 2157, **2004**.
49. LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265, **1951**.
50. VAN HANDEL E. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control Assoc. **1** (3), 302, **1985**.
51. KHAN A.L., WAQAS M., LEE I.-J. Resilience of *Penicillium resedanum* LK6 and exogenous gibberellin in improving *Capsicum annuum* growth under abiotic stresses. J Plant Res. **128** (2), 259, **2015**.
52. KHAN A.L., SHINWARI Z.K., KIM Y.-H., WAQAS M., HAMAYUN M., KAMRAN M., LEE I.-J. Role of endophyte *Chaetomium globosum* LK4 in growth of *Capsicum annuum* by producion of gibberellins and indole acetic acid. Pak. J. Bot. **44** (5), 1601, **2012**.
53. ABD-ALLAH E.F., ALQARAWI A.A., HASHEM A., RADHAKRISHNAN R., AL-HUQAIL A.A., AL-OTIBI F.O.N., MALIK J.A., ALHARBI R.I., EGAMBERDIEVA D. Endophytic bacterium *Bacillus subtilis (BERA 71)* improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. Journal of Pant Interactions. **13** (1), 37, **2018**.
54. SHINOZAKI K., YAMAGUCHI-SHINOZAKI K. Gene networks involved in drought stress response and tolerance. J Exp Bot. **58** (2), 221, **2007**.
55. HARE P., CRESS W. Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul. **21** (2), 79, **1997**.
56. RAGHAVENDRA A.S., GONUGUNTA V.K., CHRISTMANN A., GRILL E. ABA perception and signalling. Trends Plant Sci. **15** (7), 395, **2010**.
57. TOH S., IMAMURA A., WATANABE A., NAKABAYASHI K., OKAMOTO M., JIKUMARU Y., HANADA A., ASO Y., ISHIYAMA K., TAMURA N. High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant Physiol. **146** (3), 1368, **2008**.
58. SONG Y., CHEN Q., CI D., SHAO X., ZHANG D. Effects of high temperature on photosynthesis and related gene expression in poplar. BMC Plant Biology. **14** (1), 111, **2014**.
59. CHANG J., WANG Y., SHAO L., LABERGE R.-M., DEMARIA M., CAMPISI J., JANAKIRAMAN K., SHARPLESS N.E., DING S., FENG W. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat. Med. **22** (1), 78, **2016**.
60. BOERJAN W., RALPH J., BAUCHER M. Lignin biosynthesis. Annu. Rev. Plant Biol. **54** (1), 519, **2003**.
61. MITTAL S., KUMARI N., SHARMA V. Differential response of salt stress on *Brassica juncea*: photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. Plant Physiol. Biochem. **54**, 17, **2012**.
62. BIENERT G.P., CHAUMONT F. Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. Biochim. Biophys. Acta Biochim Biophys Acta. **1840** (5), 1596, **2014**.