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Primary and Secondary Metabolites of *Vicia faba* Plants Cultivated under the Interactive Effect of Drought and Nitric oxide

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IMPROVING of drought tolerance of plant is the main target of recent studies over the world. The present investigation deals with using nitric oxide (NO) as a protecting agent against drought stress. NO act a vital role in diverse physiological functions in plants. Presoaking of seeds of two faba bean cultivars (Giza 3 and El-masria 3) at NO (0.1mM) for 8 hrs. and control plants were soaked in distilled water. Plants were cultivated at three levels of water availabilities (100, 70 and 50% FC). Deleterious impact of drought was reduced of chlorophyll *a* and reduction of total protein, soluble protein in El-masria 3 cultivar. Whilst drought stress reduced secondary metabolism phenolic compounds but anthocyanins were enhanced by stress. No alleviated the bad impact of water deficiency on cultivars of faba beans by improved the chlorophyll *a*, carotenoids, soluble carbohydrates, free amino acids, phenolic compounds and anthocyanin. Exogenous application of nitric oxide (0.1mM NO) may improve the performance of faba bean to be more drought-tolerant.

.**Keywords:** Drought, Faba beans, Nitric oxide, Primary and secondary metabolites

Introduction

Plants are usually under pressure in its environmental and natural habitat due to biotic factors pathogens such as (viruses, bacteria and fungi) and abiotic factors (drought, floods, salt, heat, metals and cold). Biotic and abiotic stresses can influence on the growth, improvement and production, hence affecting global food safety (Shaaban & Maher, 2016, Nabi et al., 2019). As a response to stress, plants suffer from changes in gene regulation, metabolism, and physiology. Drought stress is the most important abiotic stresses, causing many morphological, physiological, biochemical and molecular changes in plants (Ahmed et al., 2016; El-Far et al., 2019).

Improving stress tolerance in plants has main effects in agriculture and horticulture. As a protection against stress, plants release signal molecules that begin a series of responses to acclimatize to stress leading to plant adaptation. Exogenous chemical treatments are a convenient and effective approach for enhancing the stress tolerance of crops; one of these compounds nitric oxide (NO). Many studies have described the enhanced NO production as the subsequent to drought stress in different species across the plant kingdom such as *Citrus aurantium* (Ziogas et al., 2013), *Ailanthus altissima* (Filippou et al., 2014), *Oryza sativa* (Cai et al., 2015), *Hordeum vulgare* (Montilla-Bascon et al., 2017). Thus, it could be suggested that the exogenous application on NO, at appropriate concentration, may be helpful in alleviating damaging impacts of drought stress.

Formerly, the ethylene was the only gaseous signaling molecule in the living world recognized to science. However, nitric oxide (NO) was documented in the 1998 Nobel Prize for Medicine as signal molecule in mammalian cells (Wojtaszek, 2000). Nitric oxide considered as a small diatomic molecules with highly diffusion. Also NO acting a dynamic role in varied physiological functions in plants, for example, motivation of seed germination and reduction of seed dormancy (Libourel et al.,

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2006; Zheng et al., 2009). Also, NO performances a vital role in regulation of senescence and plant metabolism (Leshem & Haramaty, 1996; Guo et al., 2005), cell death induction (Pedroso et al., 2000), controlling of stomatal movement (Sakihama et al., 2002; Neill et al., 2003; Bright et al., 2006; Garcia-Mata & Lamattina, 2001). NO influences on photosynthesis regulation (Takahashi & Yamasaki, 2002), mitochondria functionality (Zottini et al., 2002), gravitropism (Hu et al., 2005), and regulation of flowering (He et al., 2004).

Exogenous application of nitric oxide (NO) may performance a significant role in drought-tolerant plants in many crops species (Leite et al., 2019). It was exhibited that exogenous NO improved drought tolerance and improved net photosynthetic rate in wheat (Garcia-Mata & Lamattina, 2001; Boyarshinov & Asafova, 2011) and rice crops according to (Farooq et al., 2009) by improving stability of membrane, enhancing activities of antioxidant enzymes and reducing H_2O_2 and MDA contents.

Despite water deficit effect on all variables, exogenous application of nitric oxide promoted attenuation of signs and symptoms on photosynthetic performance, carbon assimilation, efficiency of water use and chlorophyll content (Leite et al., 2019). Leite et al. (2019) observed that, sugar accumulation and water potential of leaf stimulated by NO application. Also, NO reversed and/or reduced symptoms of drought on plant growth, specifically the height of the plant, leaf area and dry matter accumulation. The presence of NO donors and ROS enhanced the abscisic acid (ABA) synthesis in wheat roots under drought stress (Zhao et al., 2001). Furthermore, NO accumulation was shown to be essential in ABA caused closure of stomata in *Vicia faba* plants (Garcia-Mata & Lamattina, 2002). Exogenous application of SNP prevented drought-induced decrease in growth performance, the content of water and stability of membrane by enhancing proline accumulation and activation of antioxidant enzymes accompanied by a decrease in lipid peroxidation and H_2O_2 content under drought stress (Lei et al., 2007; Nasibi & Kalantari, 2009).

Thus, in this study, we tended to soak plants in the selected protective substance, NO, with optimum concentration 0.1mmole NO (data not shown). To investigated the response of primary and secondary metabolites to interactive between different levels of water deficit and NO in two cultivars of faba bean.

Materials and Methods

Experimental design

Seeds of faba bean cultivars (Giza 3 and El Masria 3) were soaked in the optimal concentration of NO (0.1mM) (data not shown) for 8hrs. and control plants were soaked in distilled water. Ten soaked seeds were sown at a depth of 1.5cm in a plastic pots containing 4kg clay soil. All pots were irrigated with tap water around the field capacity (100% FC) until the appearance of two true leaves (10 days) then the seedlings were thinned to six plants.

Water- and NO-soaked pots were sub-divided into two treatments:

a) Well-watered plants irrigated at field capacity (100% FC).

b) Water-deficit was conducted by decreasing the soil water availabilities to 70% and 50% FC.

Four pots/treatment were conducted. The pots were daily weighed and irrigated to restore the appropriate water level by adding the calculated amount of water up to yield production for water deficit plants. Treated plants were harvested after five weeks of stress imposition to do biochemical analyses.

Growth parameters

Length of root and shoot was determined as cm. Consequently, fresh weight of root and shoot were recorded then dried at 80°C for 48°C for dry weight determination.

Photosynthetic pigments

Chlorophyll *a*, *b* and carotenoids were extracted from fresh leaves suspended at 5mL ethyl alcohol (95%) and putted in water bath at (60-70°C). Absorbance readings were followed with a spectrophotometer (Unico UV -2100 spectrophotometer) at wavelengths (663, 644 and 452nm) using equations suggested by Lichtenthaler (1987).

Primary metabolites

Determination of soluble and total carbohydrates

The anthrone-sulphuric acid method (Fales, 1951; Schlegel, 1956) was used for the determination of carbohydrates.

Determination of soluble and total proteins

Proteins were determined using alkaline reagent solution according to Lowry et al. (1951) method.

Determination of free amino acids

Free amino acids were determined according to Moore & Stein (1948).

Determination of free proline content

Free proline content was estimated according to Bates et al. (1973).

Secondary metabolites

Phenolic compounds

Free phenolic compounds were determined according to Kofalvi & Nassuth (1995).

Total flavonoids

Methanolic extract of leaves was used for detection of flavonoids according to Zou (2004).

Anthocyanin pigments

Anthocyanin pigments were determined according to Krizek et al. (1993).

Statistical analysis

Means of three replicates of each trait were compared by Duncan's multiple range tests using one-way ANOVA (SPSS 10.0 software program) where the statistical significance was estimated at 5% level.

Results

Various responses of the two cultivars of faba beans (Giza 3 and El-masria 3) to different levels of water deficit, also the response to nitric oxide application differ in two cultivars. The results as following:

Fresh and dry weight

Data represented in Tables 1 and 2 revealed that as the water stress intensified on the soil (water deficit), increased the reduction of biomass (fresh and dry weight) at the two studied cultivars. Furthermore, the data showed that, shoots more affected than roots to water deficit stress.

TABLE 1. Shoot fresh and dry weight (SFW, SDW) gm/plant of the two faba bean cultivars (Giza 3 and El-masria 3) under water deficit stress and NO-priming

	% FC	SFW (gm/plant)		SDW (gm/plant)	
		Giza 3	El-masria 3	Giza 3	El-masria 3
0mM SNP	100	10.62±0.03 c	10.05±0.03 d	1.09±0.03 c	1.00±0.06b
	70	10.26±0.02 c	9.08±0.04 c	1.06±0.04 c	0.92±0.05 b
	50	5.70±0.04a	5.31±0.02 a	0.48±0.02 a	0.548±0.03 a
0.1mM SNP	100	14.80±0.03 d	12.36±0.02f	1.44±0.05 d	1.24±0.04 d
	70	14.79±0.06 d	11.76±0.04 e	1.74±0.05 e	1.38±0.04 c
	50	9.04±0.03 b	6.50±0.03 b	0.88±0.01 b	0.68±0.04 a

- Each value represents a mean of three replicates ± SE.
- Values carrying different letters are significantly different at P<0.05.

TABLE 2. Root fresh and dry weight (RFW, RDW) gm/plant of the two faba bean cultivars (Giza 3 and El-Masria 3) under water deficit stress and NO-priming

	% FC	RFW (gm/plant)		RDW (gm/plant)	
		Giza 3	El-masria 3	Giza 3	El-masria 3
0 mM SNP	100	6.2±0.06c	5.19±0.08a	0.96±0.02c	0.51±0.08b
	70	5.67±0.04b	5.09±0.06a	0.93±0.04c	0.50±0.06b
	50	3.6±0.03a	2.51±0.08a	0.575±0.0a	0.25±0.06a
0.1mM SNP	100	12.52±0.0e	7.52±0.04a	1.31±0.03d	0.77±0.1d
	70	12.99±0.0f	7.52±0.15a	1.31±0.02d	0.77±0.04d
	50	7.44±0.04 d	6.05±0.1a	0.752±0.0b	0.61±0.1bc

- Each value represents a mean of three replicates ± SE.
- Values carrying different letters are significantly different at P<0.05.

Of interest NO-soaking had a positive effects on shoots and roots growth parameters which varied by the water level applied or cultivar studied. NO-priming showed the highest response at the levels of 70% for cvs Giza 3 and El-masria 3 where NO soaking not only restrained the dry weight reduction via water deficit stress but also increased it above the well-watered plants. Furthermore, NO-priming at the level 50% FC completely alleviated the dry matter reduction of cv. El-Maseria 3 to be more or less comparable to control, whereas NO increased the dry weight of cv. Giza 3 compared to stressed plants only.

Photosynthetic pigments

The data histogrammed in Fig.1 revealed analysis variances of the different responses of faba bean plants to drought. The data represented in (Fig. 1) revealed that the chlorophyll *a* (Chl. *a*) of the tested faba bean cultivars exhibited antagonistic effect where increasing water deficit in growing substrate induced unexpected increased trend of chlorophyll a for cultivars Giza 3 and El-masria 3. On the other hand, Chl *b* reduced whatever the deficit irrigation level or cultivar tested.

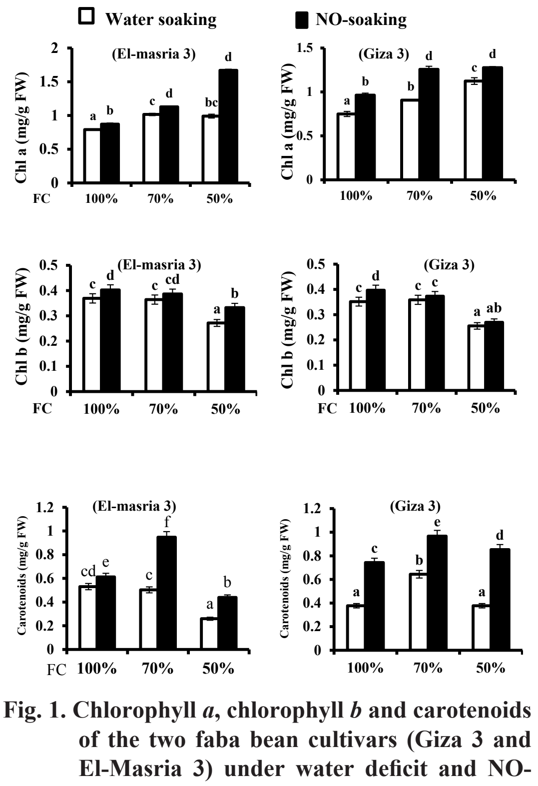

Fig. 1. Chlorophyll *a*, chlorophyll *b* and carotenoids of the two faba bean cultivars (Giza 3 and El-Masria 3) under water deficit and NO-soaking grown under different levels of water stress (100%, 70% and 50% FC) [Each value represented mean of three replicates. Bars carrying different letters are significantly different at P<0.05]

Water deficit did not alter carotenoids content of cv. Giza 3. In cv. El-masria 3 increment of carotenoids was recorded at 70% FC and then reduced dramatically for at 50% FC. Protective role of NO-priming was demonstrated whatever the water level applied or cultivars tested. Further increment of Chl *a* and carotenoids was detected for deficit irrigated plants primed with NO.

Primary metabolites

Soluble and total carbohydrates

The data displayed in Fig. 2 revealed that, soluble carbohydrates content was highly significantly accumulated for two tested cultivars under the different levels of water deficit. Total carbohydrates were increased highly significantly for cv. Giza 3 whatever the deficit irrigation level used, while did not alter in cv. El-masria 3.

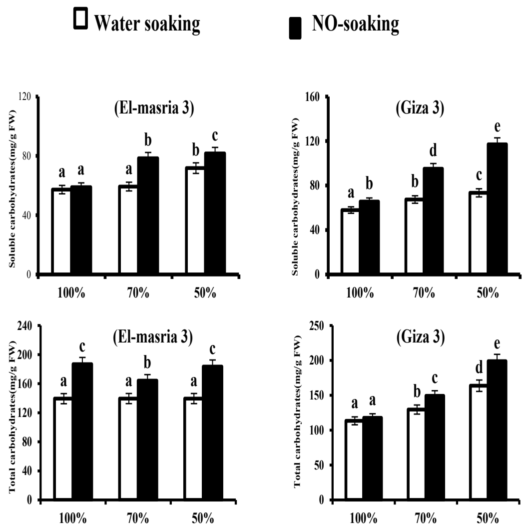

Fig. 2. Total and soluble carbohydrates of the two faba bean cultivars (Giza 3 and El-masria 3) under the interactive effect of NO-priming grown and various levels of water stress (100%, 70% and 50% FC) [Each value represented mean of three replicates. Bars carrying different letters are significantly different at P<0.05]

Pre-soaking of different cultivars with NO induced further increment of soluble and total carbohydrates accumulation, which was higher than that recorded for water deficit. The highest increasing of soluble carbohydrates content was recorded for cv. Giza 3 which pre–soaked with NO especially at 50% FC by 2.02 fold compared to control. Moreover, accumulation of the soluble carbohydrates observed in control plants which pre-soaked with NO compared to not-treated plants for almost the faba bean tested cultivars.

Total carbohydrates was increased highly significantly for cv. Giza 3 whatever the water deficit level used, while did not alter in cvs. El-masria 3. NO stimulated total carbohydrates of plants in droughted soils at two studied cultivars and all water levels.

Soluble and total proteins

Data represented in Fig. 3 revealed that, plants decreased the content of soluble proteins under water deficit especially the level of 50% FC water deficit for cv. Giza 3 where soluble proteins enhanced by water deficit at El-masria 3. The cv. El-masria 3 highly significantly increased soluble protein by about 2.26 and 2.06 fold for 70% and 50%, respectively compare to control. The data illustrated in Fig. 3 showed that, water deficit increased total proteins of cv. Giza 3, while reduced their content to be lower than control for the cultivar El-masria 3.

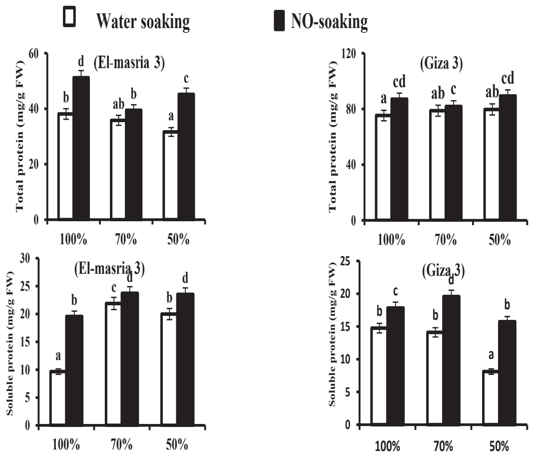

Fig. 3. Total and soluble proteins of the two faba bean cultivars (Giza 3 and El-masria 3) under the interactive effect of NO-priming grown and various levels of water stress (100%, 70% and 50% FC) **[Each value represented mean of three replicates. Bars carrying different letters are significantly different at P<0.05]**

Exogenous application of nitric oxide as pre-sowing induced furthermore accumulation of soluble protein for cv. El-masria 3 and alleviated the reduction of soluble protein by water deficit for cv. Giza where their contents generally higher than control plants. Also, the pre-sowing of seeds in NO increased total proteins biosynthesis.

Free amino acids

The data histogrammed in Fig. 4 revealed that water deficit stress reduced free amino acids content for the cultivar Giza 3. On the other hand, water deficit stimulated free amino acids for the cultivar El-masria 3.

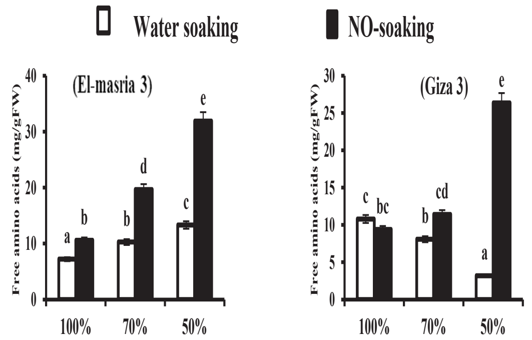

Fig.4. Free amino of the two faba bean cultivars (Giza 3 and El-masria 3) under the interactive effect of NO-priming grown and various levels of water stress (100%, 70% and 50% FC) **[Each value represented mean of three replicates. Bars carrying different letters are significantly different at P<0.05]**

Nitric oxide alleviated the reduction of free amino acids caused by water deficit for cv. Giza 3. Whilst, NO induced further free amino acids biosynthesis for cvs El-masria 3 under water deficit stress. Obviously, increased of free amino acids in control plants which treated with NO at cv. El-masria 3.

Proline

The data illustrated in Fig. 5 revealed various changed on proline content in relation to different treatments. As the water level reduced in the soil, proline content enhanced progressively to be maximally reported at the level of 50% FC of the two faba bean cultivars tested.

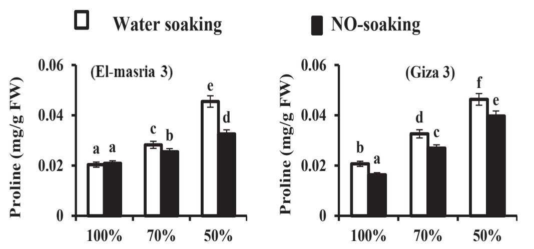

Fig. 5. Proline content of two faba bean cultivars (Giza 3 and El-masria 3) under the interactive effect of NO-priming grown and various levels of water stress (100%, 70% and 50% FC) **[Each value represented mean of three replicates. Bars carrying different letters are significantly different at P<0.05]**

Reduction of proline content was the result of NO-soaked plants exposed to water deficit treated plants. Under control conditions, SNP pretreatment had reduction effect on proline content in leaves of cv. Giza 3, however it had no significant on cv. El-masria 3.

Secondary metabolites

Phenolic compounds

The data histogrammed in Fig. 6 represented one of important secondary metabolites produced in plants, phenolic compounds. The data of water deficit stress revealed significant reduction of phenolic content for cv. Giza 3. The cultivar El-masria 3 leaves exhibited no effect of water deficit on phenolic content.

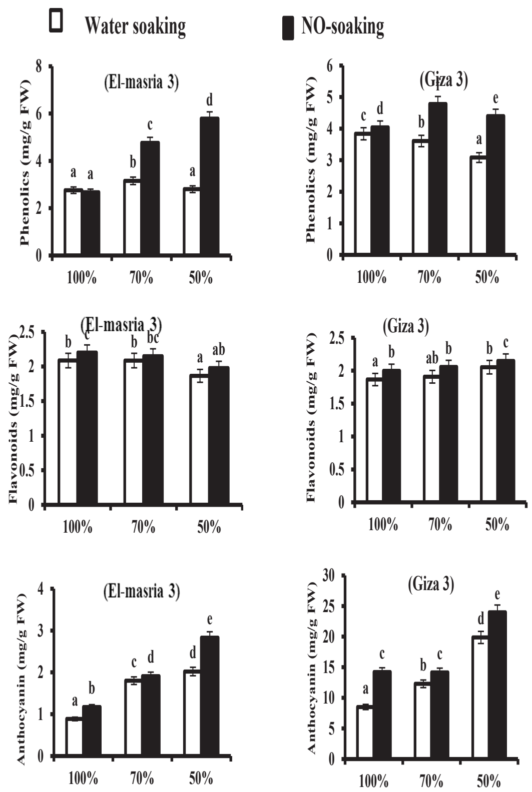

Fig. 6. Phenolic , Flavonoids and Anthocyanin content of the two faba bean cultivars (Giza 3 and El-masria 3) under the interactive effect of NO-priming grown and various levels of water stress (100%, 70% and 50% FC) [Each value represented mean of three replicates. Bars carrying different letters are significantly different at P<0.05]

The production of phenolics was highly enhanced via NO application whatever the cultivars tested or water stress dose applied. NO application not only recovered the reduction of phenolic compound content of cv. Giza 3 under water deficit stress but increased its content higher than the control. For water deficit treated NO, the level of 50% FC+NO exhibited higher accumulation of phenolic compounds at cv. El-masria 3.

Flavonoids

The data represented in Fig. 6 showed a significant accumulation of flavonoids content by water deficit stress for cv. Giza 3. Whilst, water deficit stress reduced flavonoids for cv. El-masria 3. NO had promoting effect on flavonoids content to be higher than corresponding level for two studied cultivars.

Anthocyanin

The differential effects of various treatments were represented in Fig 6. Anthocyanin enhanced progressively by water deficit stress at two studied cultivars and water deficit levels. Promotion effect of NO on leaves anthocyanin content was attained for deficit irrigation (Fig. 6).

Discussion

Water availability is an issue of great concern nowadays particularly in arid and semiarid areas like Egypt. Especially with recent global climate change which made this situation more serious. Egypt is extremely vulnerable to changes in the water supply (Oestigaard, 2010). From one hand, Egypt is listed among the top ten countries in the world which will be most threatened by water shortage in the future (Oestigaard, 2010). Thus, water protecting is becoming a decisive consideration for agriculture (Hammad & Ali, 2014).

Faba bean growth was harmfully affected by water deficit, compatible with other researches (Leite et al., 2019; Nabi et al., 2019). In these stress conditions some physiologically changes are shown (Taiz et al., 2017), which reduces growth and productivity of crop. It has been suggested that the growth inhibition caused by water deficit could partly be ascribed to the energy consumption in the processes of drought tolerance and the repairing mechanism of membranes and proteins (Behnamnia, 2015).

Because SNP is the only NO donor, that stimulates electron transport through photosystem II (Prochzkov et al., 2013) and increases ribulose-1, 5-bisphosphate carboxylase/ oxygenase activities (Fatma & Khan, 2014). Presoaking of the two tested faba bean cultivars in the desired concentration of NO was augmented stress tolerance in terms of dry and fresh matter accumulation with varying responsiveness based on the tested cultivar and water level imposed. Various studies supported the participation of NO in plant signaling in responses to abiotic stresses (Cai et al., 2015; Cechin et al., 2015; Silveira et al., 2016; Jangid & Dwivedi, 2017). Plant growth stimulation by NO application was described in some studies (Wang et al., 2016; Chen et al., 2018).

The results of this study suggested that SNP could significantly increase the chlorophyll contents at cvs. (Giza 3 and El masria) and all water levels similar to the findings (Jday et al., 2016; Leite et al., 2019). So, exogenously applied SNP improved chlorophyll levels of faba bean plants exposed to water deficit stresses by mitigating the leaf senescence process. Also, exogenous nitric oxide stimulated the increase of photosynthetic pigments in different species under abiotic stress, as tomato (Manai et al., 2014), rice (Habib et al., 2013) and chickpea (Ahmad et al., 2016). While reduced chlorophyll b. at two studied cultivars and all water levels. In leaves of *Brassica napus*, NO up-regulates the level of chlorophylls and Chl *a/b* ratio under cadmium stress (Jhanji et al., 2012). Supplementation of SNP prevents interveinal chlorosis and enhancements chlorophyll content by 70% in Fe-deficient mesophyll cells of *Zea mays* (Graziano et al., 2002). The availability of iron from the dissociation of SNP is also another option for boosted chlorophyll content and PSII electron transport rate. In the present research, NO-donor priming exacerbated photosynthesis on drought stressed plants. SNP-treated leaves have highly chlorophyll content in comparison with their corresponding controls, which is likely due to improved iron uptake and availability in the treated plants and that through the dinitrosyl iron complexes formation (Liao & Huang, 2012; Koen et al., 2012).

In the present investigation, NO priming enhanced the development and growth of faba bean plants via improving carbohydrates metabolism which have the vital importance in plant construction and providing the cells with energy necessary to maintain better growth under drought. Thus, the accumulation of carbohydrates under the interactive effect of NO and different water levels may be accompanying with the increased photosynthetic rates under both stresses as was observed (Tanou et al., 2009, 2012; Molassiotis et al., 2016; Neves et al., 2018). The increment of carbohydrates in the tissues may be represented as osmoprotectant molecules mainly in the H_2O_2 scavenging processes (Neves et al., 2018; Akhtar & Nazir, 2013).

Regarding the drought stressed plants; most the cultivars increased both chlorophyll and soluble carbohydrates. But, such increment of chlorophyll did not match similar accumulation of total carbohydrates.

a. In the sensitive cultivar (El-masria 3), the increment of chlorophyll went in parallelism to increased soluble carbohydrates, but no change of total carbohydrates. Thus, the insoluble content reduced for these sensitive cultivars; thereby the enhancement of soluble carbohydrates for these cultivars was used only for osmo-protectant. Similarly, soluble sugars have been considered as osmotic regulators and of which non-reducing sugars, such as disaccharides and oligosaccharides, are the carbohydrates most directly involved in membrane stability, while high reducing sugars levels are accompanying with high metabolism and a loss of desiccation tolerance (Pence et al., 2005). Thorpe et al. (1999) and Valluru (2015) stated that soluble carbohydrates in high concentrations have often been found to be limiting organ growth. However, the low levels of soluble carbohydrates may be temporarily increase carbon require and thus improve carbon discharge of the phloem.

b. In the tolerant cultivar Giza 3, drought stress amplified the chlorophylls, soluble carbohydrates and total carbohydrates, hence insoluble carbohydrates. A main role of sugars depends not only on direct association with the synthesis of other compounds, production of energy but also on membranes stabilization (Hoekstra et al., 1996). Also as regulators of gene expression (Koch et al., 1996) and as signal molecules (Smeekens, 2000). Carbohydrate performed several functions as osmoprotection, osmotic adjustment, carbon storage and detoxification of ROS. In addition to protection of macromolecules and DNA structures, stabilization of enzymes and proteins and protection of membrane integrity (Ahmad et al., 2008; Chehab et al., 2009).

Variation of protein content is an important part of plant response to environmental stress as well as for adaptation to changes in environmental conditions, under conditions of water availabilities numerous processes are modified or impaired. Soluble protein increased by water deficit in cv. El-masria 3 but soluble protein decreased at cv. Giza 3. The alternation of protein synthesis is one of the major of the fundamental metabolic processes that influenced by different irrigation levels. In the stressful conditions, plants modify their metabolism and synthesize metabolites to adjust their homeostasis (Siddiqui et al., 2008).

In the present investigation, all the tested cultivars used soluble carbohydrates as osmolyte under the different levels of water-deficit stress, but the utilization of nitrogenous components (proteins, amino acids and proline) were different.

In the case of cultivar Giza 3: the increment of the total proteins and proline accompanied with reduction of soluble proteins and free amino acids. This revealing that these cultivars sufficed with soluble carbohydrates and proline as osmo-regulators. In the case of cv. El-masria 3 (the most drought sensitive cultivar): the cultivar reduced the total proteins in favor of free amino acids and proline. The current results are in accordance with Al-Jebory (2012) that water stress induced proteins allow plants to make biochemical and structural adjustments that enable plants to cope with the stress. An induction of soluble proteins under drought stress was reported for (Mafakheri et al., 2011; John et al., 2011; Wegener et al., 2014).

Nitrogenous compounds such as free amino acids, proline, glycine betaine and polyamines play important roles in the maintenance of water uptake capacity from dry substrate and protection of tissues from stress damages (Verbruggen & Hermans, 2008).The data provided in this study, under severe applied stress (50% water deficit) that free amino acids was depleted compared to normally irrigated plants. Similar reduction of free amino acids was registered by Hammad & Ali (2014) on wheat as a consequence of deficit irrigation. Proline has been well documented as “an osmotic regulator” assisting in reduction of osmotic damage (Anee et al., 2019). Proline accumulates in many plants in response to wide range of biotic and abiotic stresses and a well-known as osmo-protectant (Zhang et al., 2016; Leite et al., 2019). However, the application of SNP, herein, differentially affected proline accumulation drought stress.

Under the interactive effect of drought and NO, proline content was retarded comparing with the corresponding drought level, implying that NO could improve water status and up-regulate metabolic pathways of plants under drought stress by triggering other osmolytes. The reduction of proline content was due to increased activity of proline dehydrogenase induced by NO (López-Carrión et al., 2008). Ke et al. (2013) reported that NO application decreased the cell solutes and increased the water potential, and thus osmoregulation of tobacco callus improved under osmotic stress.

The phenylpropanoid pathway is one of the most important pathways of plant secondary metabolism, which produces a variety of phenolics with structural and defense-related functions (Sallam et al., 2019).

The antioxidant properties of phenolic and flavonoid compounds are mediated by scavenging radical species (El-Beltagi & Mohamed, 2013). Also they can be suppressing (ROS/RNS) formation via inhibiting of activity of some enzymes. Thus, NO priming mediated drought tolerance of both cultivars by means of further production of phenolics at two studied cultivars that could participate clearly for reduction ROS, hence membrane integrity. Phenolic compounds have antioxidant properties that can avoid plants from oxidative stresses (Wang et al., 2010).

Anthocyanins as a water-soluble pigment belong to phenolic compounds. Higher leaf concentration of pigments particularly anthocyanin may be important for increasing plant tolerance to various stress conditions. It has been described that plant tissues containing anthocyanin are usually resistant to drought that is associated with superoxide radical scavenging activity and of anthocyanins capability to stabilize the water potential (Pazirandeh et al., 2013; Kovinich et al., 2015). Thus, the two studied faba bean cultivars, herein, induced higher anthocyanin levels comparing with control.

Furthermore, anthocyanins are thought to act as osmoregulators under drought stress, because many drought-tolerant plant species contain anthocyanins (Chalker-Scott, 1999). Kovinich et al. (2015) showed that anthocyanins produced in *Arabidopsis* under abiotic stresses have various localizations at the organ and tissue levels. Nakabayashi et al. (2014) reported that flavonols and anthocyanins can alleviate drought stress.

Unequivocally, NO-priming greatly up-regulated shykimic acid pathway products, thus anthocyanin was found to be accumulated under both stresses higher than the stressed plants only. In a way to increase the protection of plants against reactive oxygen species and membrane deteriorations (Kovinich et al., 2015; Costa-Broseta et al., 2018; Xiaolan et al., 2018).

Conclusion

Presoaking of faba bean cultivars in the desired concentration of NO increased stress tolerance in terms of dry and fresh matter, carbohydrates, phenolic compounds, flavonoids and anthocyanin accumulation with varying responsiveness based on the tested cultivar, water level imposed and even at the organization level.

Conflict of interest: The authors reported no potential conflict of interest.

Authors contribution: Zidan, Design the experiments and write the manuscript, Aldaby, Co-design the experiments, participated in writing manuscript and publishing the research, Dawood, Co-design the experiments and participated in writing manuscript, Dief, did the practical part and participated in writing manuscript.

Ethical approval: Not applicable.

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التأثير التفاعلي للجفاف وأكسيد النيتريك على مركبات الايض الأولية والثانوية لنبات الفول

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تحسين مقاومه النباتات للجفاف هو الهدف الرئيسي للدراسات الحديثة في جميع أنحاء العالم. يتناول التحقيق الحالي استخدام أكسيد النيتريك كعامل وقائي ضد إجهاد الجفاف. يلعب أكسيد النيتريك دورًا حيويًا في الوظائف الفسيولوجية المتنوعة في النباتات. قمنا بنقع بذور صنفين من الفول (الجيزة 3 والمصرية 3) عند تركيز (0.1 مم من اكسيد النيتريك) لمدة 8 ساعات وتم رى العينات الغير معامله بالماء المقطر. تم زراعة النباتات على ثلاثة مستويات من المياه (100 ، 70 و 50 % من السعه الحقليه). تم تقليل التأثير الضار للجفاف من تقليل الكلوروفيل أ. وخفض البروتين الكلي والبروتين القابل للذوبان في أصناف المصرية. في حين أن إجهاد الجفاف قلل من المركبات البروتينيه في الأيض إلا أن الإجهاد حسن محتوى الأنثوسيانين. وقام اكسيد النيتريك بتخفيف الآثار السيئة لنقص المياه على نبات الفول وذلك عن طريق تحسين كل من Chl *a* ، الكاروتينات ، الكربوهيدرات القابلة للذوبان ، الأحماض الأمينية الحرة ، المركبات الفينولية والأنثوسيانين.