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PHOTOSYNTHETIC PERFORMANCE AND ACTIVITY OF ANTIOXIDANT ENZYMES INDUCED BY SEED PRIMING IN MAIZE PLANTS

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ABSTRACT

Maize plants are often exposed to unfavourable environmental conditions - abiotic factors which limits their development and productivity. Priming of seeds pre-sowing is a low cost, low risk and very simple method for the farmers to improve germination, seedling emergence, growth and yield of plants especially under adverse environmental conditions.

The present study revealed effects of different seed priming treatments on proline, total phenols, carbohydrates, chlorophylls and carotenoids content and antioxidant enzyme activities of maize leaves.

The results showed that priming with basil oil, $CaCl_2$ and SA increased chlorophylls and carotenoids content improved tolerance to abiotic stress of maize plants. However only seeds treatment with SA and $CaCl_2$ increased activities of antioxidant enzymes (CAT, POX and PPO). These effects were significantly positively correlated with the accumulation of proline and soluble sugars, which can alleviate oxidative membrane damage increased stress tolerance of maize plants. The results suggest that SA and calcium chloride application enhanced stress tolerance in maize plants and could be involved in the scavenging of ROS by increasing SOD and CAT activities. The study revealed that pre-sowing seeds treatments can be successfully used to improve the performance of different maize cultivars/hybrids by increasing the physiological resistance to abiotic stresses.

Keywords: chlorophylls, proline, physiological resistance, basil oil, bay oil, $CaCl_2$ and salicylic acid seed priming.

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop worldwide after wheat and rice. Maize crop is sensitive to different abiotic stresses, which may cause more than 50% yield reduction.

Abiotic stresses such as heat, cold, excess moisture and salinity are the most important environmental stresses which can cause morphological, physiological, biochemical changes that severely affect crop production in many areas of the world. The response of plants to abiotic stress vary according to species, severity and duration of the stress, as well as the plants growth stage.

Plants tend to adapt to different abiotic stressors by accumulating specific compounds or by increasing the antioxidant defense system. Improving stress tolerance in maize has become one of the top priorities for maize breeding programs in research sectors (Farooq et al., 2008; Kaya et al., 2010; Bakht et al., 2012; Khan et al., 2015; Moharramnejad et al., 2019). Identification of maize germplasm with superior tolerance is essential and prerequisite for such propose.

It is important to develop practical and efficient technologies to mitigate the negative effects produced by abiotic stressors on plant growth and development. Development of stress-tolerant plant varieties will play an important role in alleviating the negative impacts of abiotic stresses on agricultural production. Several ways and means for enhancing the plant tolerance towards abiotic stress have been experimented, like breeding of plants and developing transgenics (Jisha et al., 2013).

The external application of some biologically active compounds (salicylic acid, calcium chloride, essential oils, vegetable

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extracts, mannitol, b-amino butyric acid, thioureea) by the treatment of seeds or foliar can be an alternatives to increase yield of different crops and tolerance to different abiotic stress factors by improving the antioxidant defense system, increase of different metabolites with resistance role and stimulating the physiological resistance (Asharf and Foolad, 2007; Kaya et al., 2010; Ahmad et al., 2012; Agami, 2013; Jisha et al., 2013; Rehman et al., 2015; Szalai et al., 2016; Jisha and Puthur, 2016, 2018; Farooq et al., 2018).

The positive role of salicylic acid (SA), calcium chloride ($CaCl_2$) in improving stress tolerance has been demonstrated in different crops (Gunes et al., 2007; Farooq et al., 2008; Hayat et al., 2010; Syeed et al., 2010; Saruhan et al., 2012; Al-Whaibi et al., 2012; Khan et al., 2015; Kaczmarek et al., 2017; Zanganeh et al., 2019) by inducing a wide range of stress acclimation mechanisms by activating the defense system.

Previous studies reported that tolerant genotypes have higher levels of antioxidants in their tissues than sensitive ones. Plants can upregulate their antioxidative defense mechanism by stimulating the activities of key antioxidative enzymes including superoxide dismutases (SOD), catalases (CAT), peroxidases (POD) and polyphenol oxidase(PPO) (Ashraf, 2009; Al-Whaibi et al., 2012).

The accumulation of osmolyte compounds in the cells of plant as a result of stress is often associated with a possible mechanism to tolerate the harmful effect of different deficiencies. Also, osmolyte accumulation in plant cells might contribute to maintaining several physiological processes, such as photosynthesis, stomatal conductance, and leaf expansion even under stressed conditions (Chechin et al., 2006; Moharramnejad et al., 2019).

The aim of this study was to evaluate effects of seed priming with salicylic acid, calcium chloride, basil oil, bay oil and water on photosynthetic pigments, antioxidant enzymes, total sugars, proline and total phenols in maize plants.

MATERIAL AND METHODS

The seeds of maize hybrid P9486 were obtained from Turda Agricultural Research and Development Station. Maize seeds were surface sterilized in 2% (w/v) hypochlorite solution for 10 min and after in 70% (v/v) ethanol solution for 10 minutes, rinsed several times in sterilized distilled water and transferred to flask for priming.

For priming, seeds were pretreated/soaked for 20h at a 1:5 (w/v) ratio with calcium chloride ($CaCl_2$) 0.1% solution, salicylic acid (SA) 0.01% solution, bay oil and basil oil 5% solution and distilled water, while the untreated dry seeds were taken as controls. Concentration, time and ratio were optimized in preliminary experiments (Petrişor et al., 2017). All primed seeds and untreated seeds were air dried at 25°C for 48 h to their original moisture content, then sown in pots filled with sterile soil.

Physiological parameters such as total chlorophyll and carotenoids, total soluble sugar concentration, proline content, total phenols, peroxidase (POD), catalase (CAT) and polyphenoloxidase (PPO) activity were measured in leaves of maize plants at 60 (DAS) day after sowing stages.

Chlorophyll (Chl a, b*)* and total carotenoid (Car) contents were determined and calculated according to Lichtenthaler and Wellburn (1985) method. In brief, leaves photosynthetic pigments were extracted with 80% (v/v) acetone in presence of calcium carbonate. Extracts were centrifuged at 5000 rpm for 15 min and the absorbance of the supernatant was measured at 663, 646 and 470 nm with a UV - VIS spectrophotometer. Chlorophylls and carotenoids contents were calculated by specific formulas and results expressed as mg g^{-1} fresh weight.

The proline content was assayed by method of Bates et al. (1973). The filtrate obtained by grinding a fresh leaves sample in sulfosalicylic acid (3%) was reacted with acid-ninhydrin solution and glacial acetic acid. The mixture was placed in a water bath at 100°C for 60 min and then at reaction mixture was added toluene and OD recorded at 520 nm. Results were expressed as mg g^{-1} fresh weight.

Total phenolic content was determined from 70% ethanol extract of leaves by using Folin-Ciocâlteu reagent (Singleton and Rossi, 1965). Total phenolics express as mg phenols (in terms of catechol) per grams fresh tissue.

Total soluble sugars were assayed using the phenol sulfuric acid method (Dubois, 1956). Soluble sugars were extracted from 0.1 g fresh leaves by boiling in 80% ethanol for 15 min on water bath. Extracts were centrifuged at 2500 rpm for 5 minutes for clarification and supernatant decanted and the sediment re-extracted twice.

To assay enzyme activities, fresh leaves tissue from each treatment were homogenized in an ice cooled mortar in 100 mM Potassium - phosphate buffer, pH 7.0 containing 1% soluble polyvinyl polypirolidone (PVP) and 1 mM EDTA. The homogenate was centrifuged at 10 000 g for 15 min and the supernatant used for assay of the antioxidant enzymes activity.

Catalase (CAT) activity was measured according the method of Beers and Sizer (1952). The reaction mixture consisted of 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 20 mM H_2O_2 and 50 μL enzyme extract. The reaction was started by the addition of extract. The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 $M^{-1}cm^{-1}$) and the results expressed as μmol H_2O_2 min^{-1} g^{-1} fresh weight. One unit of CAT was defined as the amount of the enzyme causing the decomposition of 1 μmol of H_2O_2 per min.

The peroxidase (POD) activity was estimated according to the method of Hammerschmidt et al., (1982) using guaiacol as the substrate. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 5 mM H_2O_2 and 13mM guaiacol. The reaction was initiated by adding peroxidase extract at 25°C. The tetraguaiacol formed in reaction was followed spectrophotometrically at 470 nm. One unit of POD was defined as the amount of enzyme which produces 1.0 absorbance change at 470 nm per min.

Polyphenol oxidase (PPO) was determined according to the method described by Mayer et al. (1966).

The assay of the enzyme activity was performed using 0.1 M sodium phosphate buffer (pH 7.0), 100 mM catechol, and enzyme solution. The increase in OD at 420 nm at 25°C was recorded continuously for 5 min. One unit of enzyme activity was defined as an increase of absorbance at 420 nm per minute.

Collected data regarding all parameters were analyzed by using analysis of variance (ANOVA) to compare the differences among treatment means.

RESULTS AND DISCUSSION

Exposure of maize seeds to some priming solutions induced different physiological and biochemical changes that can related to stress tolerance. The maize plants studied in this experiment exhibited physiological differences in response to different seed priming treatments. Priming of maize seeds with basil oil, calcium chloride and salicylic acid caused an increase in the leaf photosynthetic pigments content of the maize plants compared with untreated control (Table 1).

On the other hand, the increase in the carotenoids content of the maize leaves treated with calcium chloride (1.03 mg g^{-1} FW) was equal with hydropriming (1.04 mg g^{-1} FW) and nearly equal with salicylic acid treatment (0.94 mg g^{-1} FW). The basil oil treatment was more effective than the treatment with calcium chloride and salicylic acid on chlorophylls and carotenoid content of leaves. According to Tabrizi et al. (2011), seed priming causes an increase in leaf chlorophyll content in maize seedlings. The increased amount of these pigments in turn resulted in effective absorption of light energy into the photosystems, resulting in an enhanced photosynthesis activity of the maize plant raised from primed seeds. Treatment of the seeds with bay oil seems to have a lower effect on the assimilating pigments content compared to the other treatments, but higher compared to the untreated control.

The chl a/chl b ratio used as a stress indicator increased in all priming variants compared to the untreated control. The values of this ratio varied between 2.5 and 3 in the treated variants, which represent optimal values for the proper functioning of the photosynthetic apparatus.

The treatment of maize seed with SA and calcium chloride strongly increased photosynthetic pigments and overcame the oxidative stress generated by different abiotic factors. Increase of total chlorophylls and carotenoids might be explained by the fact that SA and $CaCl_2$ had a protective effect on the leaf, preventing chloroplast degradation.

Our results agree with the previous studies of Khodary (2004) and Agami (2013), which indicated that salicylic acid seed treatments increased the content of photosynthetic pigments in maize leaves. However, Zanganeh et al. (2019) claimed that seed pretreatment with salicylic acid had no significant impact on chlorophylls content. Also, Anandhi and Ramanujam (1997) stated that soaking the seeds of *Vigna mungo* in solutions of SA (10-150 μM) lead to a decrease in the content of chlorophyll and carotenoid in the leaves of plant.

Table 1. Photosynthetic pigment content in maize leaves after seeds were treated with different priming solutions

Seed priming treatment	Chl a mg g^{-1} FW	Chl b mg g^{-1} FW	Chl a / Chl b	Total chl mg g^{-1} FW	Carotenoids mg g^{-1} FW	Carotenoids/ total chl
M1 (untreated control)	3.20±0.07	1.37±0.5	2.34	4.57	0.78±0.8	0.17
M2 (hydropriming)	4.83±0.08	1.62±0.6	2.98	6.45	1.04±0.5	0.16
V1 (bay oil 5%)	4.23±0.09	1.41±0.5	3.00	5.64	0.86±0.8	0.15
V2 (salicylic acid 0.01%)	4.62±0.08	1.73±0.6	2.67	6.35	0.94±0.9	0.15
V3 ($CaCl_2$ 1%)	5.36±0.05	1.84±0.7	2.91	7.20	1.03±0.5	0.14
V4 (basil oil 5%)	6.93±0.05	2.30±0.8	3.01	9.23	1.36±0.4	0.15

The data are expressed as means ±SDV (standard deviation) of three replicates in each experiment.

The data herein obtained revealed that soaking maize seeds in SA, basil oil and water stimulated the accumulation of total soluble sugars as compared to control. However, maize plants whose seed were treated with calcium chloride showed highest total soluble sugars content in comparison with control plants. Also, basil oil, salicylic acid and water induced similar content of soluble sugars in leaves (43 mg g^{-1} FW) and thus had the same effect on inducing tolerance stress. Some other studies (Mahboob et al., 2015; Mandany and Khalil, 2017) reported that osmopriming with $CaCl_2$ induced a stimulatory effect on the accumulation of soluble sugars as compared with reference control in sunflower and maize plants. In rice and wheat, total soluble sugar content increased in the seedlings raised from hydro and haloprimed seeds (Nawaz et al., 2013). In another study Farooq et al. (2018) stated that seed priming with calcium chloride was beneficial for improvement in root/shoot dry weight in maize. Probably priming with $CaCl_2$ enhance the seed Ca^{2+} contents improving carbohydrate metabolism.

Proline is considered as one of the important parameters that can be used for screening of plants for resistance to abiotic stress. Proline acts as a source of organic nitrogen reserve, osmoprotectant and antioxidant under stress and seed priming solutions may have stimulatory effect on proline accumulation (Ali et al., 2013). The intensity of proline accumulation revealed the tolerance level of plants. The variety of maize studied differentially responded to seed priming in the accumulation pattern of proline content. Proline leaf accumulation occurred in all the treatments (Table 2), but little quantity was observed in control. An increase in proline content of maize leaves was detected following calcium chloride and salicylic acid seed treatment compared to the untreated control (Table 2). Positive influence of salicylic acid seed priming on the increase of proline content in maize plant was also found by Zanganeh et al. (2019). Proline accumulation in rice seedlings raised from primed seeds has already been reported to increase the stress tolerance by Nawaz et al. (2013). Similar quantity of proline (0.32 mg g^{-1} FW) was found in maize plants after seeds were treated with bay oil, basil oil and water. Increase in chlorophyll and proline contents reflects the role of seeds priming in osmoregulation, and photosynthesis.

Table 2. Influence of different seed priming treatments on biochemical compounds content of maize leaves

Seed priming treatment	Total soluble sugars (mg g^{-1} FW)	Total phenols (mg g^{-1} FW)	Proline (mg g^{-1} FW)
M1 (untreated control)	30.2±0.03	16.06±0.40	0.21±0.21
M2 (hydropriming)	43.8±0.05	17.90±0.42	0.33±0.25
V1 (bay oil 5%)	39.3±0.06	16.10±0.49	0.32±0.31
V2 (salicylic acid 0.01%)	43.4±0.05	17.78±0.45	0.37±0.18
V3 ($CaCl_2$ 1%)	45.2±0.07	18.56±0.50	0.40±0.19
V4 (basil oil 5%)	43.9±0.06	17.33±0.35	0.34±0.24

The data are expressed as means ±SDV (standard deviation) of three replicates in each experiment.

Similarly, phenolic compounds associated benefits for alleviating the damaging effects of abiotic stresses, were reported (Khan et al., 2015). Phenols involved in antioxidant system, could also play direct antioxidative function due to their radical-scavenging properties.

Our experiments showed that there was no significant difference in the total phenols content between the untreated control and the variants treated by seed priming. However, calcium chloride seed treatment (18.56 mg g^{-1} FW) showed a more pronounced accumulation of total phenols compared with the untreated control (16.36 mg g^{-1} FW), this being followed by the variant treated with salicylic acid and then by the variant treated with basil oil (Table 2). Higher content of phenols in leaves with high POD activities in comparison to control indicated that content of phenols was not the limiting factor for POD activity. Soaking the seeds in bay oil and basil oil did not induce a significant change in the total phenols content of the maize leaves compared to control. Our results agree with those of Mandany and Khalil (2017) which clearly demonstrated that application of $CaCl_2$ to sunflower seeds slightly increased the amount of soluble phenols in plants.

Table 3. Influence of different seed priming treatments on antioxidant enzymes activity of maize leaves

Seed priming treatment	Catalase (U min^{-1} g^{-1} FW)	Peroxidase (U min^{-1} g^{-1} FW)	Polyphenoloxidase (U min^{-1} g^{-1} FW)
M1 (untreated control)	28.3±0.55	0.9±0.50	0.36±0.21
M2 (hydropriming)	29.7±0.55	1.1±0.20	0.44±0.31
V1 (bay oil 5%)	30.5±0.39	1.3±0.15	0.39±0.40
V2 (salicylic acid 0.01%)	40.3±0.37	2.3±0.30	0.60±0.35
V3 ($CaCl_2$ 1%)	32.1±0.45	1.5±0.18	0.50±0.33
V4 (basil oil 5%)	30.7±0.40	1.3±0.13	0.40±0.36

The data are expressed as means ±SDV (standard deviation) of three replicates in each experiment.

The results obtained show differences for CAT, POD and PPO activities in unprimed control and seed priming treatments. The antioxidant enzyme activities increased in response to the priming treatment (Table 3).

In the present study, the peroxidase activity varied and increased depending on seed treatment applied. The activity of peroxidase (POD) increased significantly (2.3 U min^{-1} g^{-1} FW respectively 1.7 U min^{-1} g^{-1} FW) in the leaves of maize plant which were raised from salicylic acid and calcium chloride primed seeds. Also, a slight increase of POD activity was found in the leaves of plants which were raised from essential oils primed seeds compared to untreated control.

The POD activity was found to be increased in the maize plant from salicylic acids primed seeds, while the enzymatic activity was found to be decreased in the plants which were obtained from essential oils seeds priming, these being more sensitive to abiotic stress.

The salicylic pretreatments caused a significant increase in the activities of CAT and POD, as compared to the untreated control. There was not a significant difference in POD, CAT and PPO activities among seeds treatments with essential oils. No significant changes in POD activity (1.3 U min^{-1} g^{-1} FW) was observed in plants obtained from seeds treated with essential oils and control. The activity of all enzymes studied was very close between plant obtained from seed hydroprimed and unprimed seeds.

Plants from untreated seeds generally had the lowest enzyme activity of PPO, POD and CAT.

Activities of different antioxidant enzymes are known to increase in response to abiotic stress. Higher POD and CAT activities help plants in defense to possible oxidative damage. Azevedo-Neto et al. (2006) suggested that an increase in the antioxidant enzymes helps plants maintain their growth under stress and may be regarded as indicators of salinity tolerance. According to Al-Whaibi et al. (2012) seed priming with SA and $CaCl_2$ significantly enhanced the antioxidant enzymatic activities but also yield.

CONCLUSIONS

The treatment with basil oil and salicylic acid were found to be superior to the other types of seed treatments in increasing photosynthetic pigments in leaves of maize.

Soluble sugars and proline content determined as general metabolic indicators implied in stress protection were also improved by seed priming with $CaCl_2$ and salicylic acid.

Salicylic acid and $CaCl_2$ pretreatments induced antioxidant enzyme activities of CAT and POD to a greater extent than the untreated control. These suggested that seed pretreatment leads to improving tolerance to stress of the tested maize hybrid.

The increase in photosynthetic pigments and a higher antioxidant enzymes activity of maize plants raised from seed pretreated as compared with control may cause higher tolerance to abiotic stress.

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