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IMPACT OF CHEMICAL PRIMING ON ROOT INFECTING FUNGI, PHOTOSYNTHESIS AND YIELD COMPONENTS OF COWPEA AND MUNG BEAN

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Abstract

The aim of the present research is to provide information regarding the effect of signal molecule (H_2O_2) and a growth hormone (SA) on growth of cowpea (*Vigna unguiculata* [L.] Walp) and mung bean (*Vigna radiata* L.) plants under lab and in field conditions. Three different concentrations of SA and H_2O_2 (0.5, 1 and 2%) were prepared and subsequently seeds were primed with the above concentrations for 5 mins. and placed on Petri plates against root infecting fungi. Results revealed that the seeds primed with 0.5% showed germination while 2% concentration inhibited the growth of *Rhizoctonia solani* Kuhn, *Fusarium oxysporum* Schlechtendahl and *Macrophomina phaseolina* (Tassi) Goid by displaying zone of inhibition. In field, shoot length and weight, root length and weight, nodules, number, length and weight of pods were increased when cowpea and mung seeds primed with H_2O_2 at 0.5% concentration. However, root infecting fungi like *M. phaseolina, R. solani* and *Fusarium* species was also decreased by application of H_2O_2 at 0.5% as compared to other treatments. Increased Fv/Fm was recorded in cowpea and mung bean seeds primed with water while increased performance index was obtained in seeds primed with H_2O_2 resulting in improved chlorophyll performance. However, seeds primed with water showed decrease in performance index.

Key words: Crop growth, Hydrogen peroxide and Salicylic acid, Performance index, Rotting fungi.

Introduction

Seed priming, considered as most practical method, improved seed performance particularly emergence and germination in seeds of many crops (Chiu *et al*., 2002). A hydration process related to soaking crop seeds in low water potential solution, re-drying after which metabolic activities proceeds but prevents radical emergence, is regarded as seed priming. However, primed seeds should be used immediately after priming because priming process retarded the storage life of primed seeds (Basra *et al*., 2003; Bradford, 1986). Many recent researches showed that priming could increase germination traits, improve seedling establishment, accelerate germination, and enhance plant growth (Bailly *et al*., 2000; Hussain *et al*., 2006; Pirasteh-Anosheh *et al*., 2011). Priming of seeds using chemical compounds has been found to improve plant tolerance in different crop and non-crop species against various abiotic and biotic stresses which are individually applied. Various molecules having prospective for priming agent against different biotic stresses which includes hormones (e.g., salicylic acid), amino acids (e.g., proline), reactive oxygen–nitrogen–sulfur species (RONSS), and even water (i.e., hydropriming) (Casenave & Toselli, 2007; Islam *et al*., 2009; Tanou *et al*., 2009; Christou *et al*., 2014; Li *et al*., 2014; Zheng *et al.,* 2018). Some of these agents induce effective result in plant tolerance. The endogenous plant molecules like NO, H_2S, H_2O_2, Mel, and PAs (or their functional analogs) have regulatory functions in plant abiotic stress tolerance (Molassiotis & Fotopoulos, 2011; Calderwood & Kopriva, 2014; Minocha *et al*., 2014; Arnao & Hernández-Ruiz, 2014). If the concentration of plant molecules become low than these molecules show exogenous applications initially, resulting an increase in their endogenous concentrations, but without consequent reduction of plant growth (Wahid *et al*., 2007; Li *et al*., 2012; Li *et al*., 2013; Shi *et al*., 2010; 2014). Recent research showed that endogenous concentration of plant molecules in plants (without priming) have been treated to different abiotic stress resulted in reduction of plant growth (Hu *et al*., 2012; Fu *et al*., 2013; Christou *et al*., 2014). Different chemical agents used as pretreatment of plants resulted in mild stress which leads to increase tolerance when the plant is exposed to an abiotic challenge. Osmo and hydropriming with sodium chloride resulted in 50% emergence, mean emergence time, plant population, higher final emergence, energy of emergence, achene yield, yield contributing factors and achene proteins (Hussian *et al*., 2006).

A technically useful tool for the management of plant stress is chemical priming by chemical agent which should be applied at very low concentrations to achieved effective result but at higher concentrations can be deleterious like NO and H_2S gave inhibiting effects on the mitochondrial electron transport chain (Sagor *et al*., 2013; Xu *et al*., 2010; Sathiyaraj *et al*., 2014; Cooper & Brown, 2008).

Low yields of crops are considered to be due to soil-borne diseases which normally damage the whole plants and products resulting in lower or no market value. According to Khan *et al*., (2009), plant diseases are globally responsible to 26% of yield loss and sometimes there may be complete crop failure. Some of the most important soil-borne diseases that are ‘soil inhabitants (fungi, bacteria, plant parasitic nematodes and viruses as well) caused by broad host ranges pathogens and produce long lived survival structures (Baysal-Gurel *et al*., 2012). The loss caused by fungal pathogens including species of *Fusarium*, *Rhizoctonia*, *Sclerotinia*, *Verticillium*, and *Macrophomina phaseolina*, are billions of dollar each year. Most important soil-borne fungal pathogens are *Fusarium solani* and *R. solani*, present in soils, causing a wide range of root rot diseases and damping- off in a wide range of vegetable and crop plants (Szczechura *et al*., 2013). *Macrophomina phaseolina* causes charcoal rot disease, cosmopolitan in arid region (Ijaz *et al*., 2013). It produces disease on over 500 plant species and causes dry root rot/stem canker, stalk rot or charcoal rot (Khan, 2007).

Mostly soil-borne pathogens are difficult to control. Different conventional strategies were applied for this purpose to maintain the productivity of soils under sustainable and organic farming systems. For this growers adopt strategies like use of resistant cultivars, biological control, crop rotation, application of organic amendments and synthetic pesticide (Patel & Anahosur, 2001; Dawar *et al.*, 2018). On the basis of above strategies, it is tempting to speculate that the selected chemicals like salicyclic acid and hydrogen peroxide might be helpful in reducing infection of root infecting fungi by producing resistant against fungal pathogens. Objectives of this research were to determine the effect of chemicals on cowpea and mung bean seed germination, growth, physiological parameters and on the colonization of root infecting fungi.

Materials and Methods

Concentrations of chemicals: The chemicals, hydrogen peroxide (H_2O_2) and salicylic acid (SA) were obtained from the laboratory of Department of Botany, University of Karachi. Three different concentrations of SA and H_2O_2 were prepared (0.5, 1, 2%) by adding sterilized distilled water in respective solution while 0% used as control and hydropriming.

***In vitro* assay:** Seeds of cowpea (*Vigna unguiculata* [L.]Walp) and mung bean (*Vigna radiata* L.) were obtained from local market of Karachi and were sterilized with sodium hypochlorite solution for 5 minutes and dry. Tested seeds were than primed with 0.5% SA and H_2O_2 for 5 minutes and allow drying aseptically. Melted Potato Dextrose Agar (PDA) was poured (15-20 mL) in Petri plates (9 mm) containing antibiotics and allowed to solidify medium. A disc of root infecting fungi (5 mm) like *M. phaseolina*, *R. solani* and *F. oxysporum* was inoculated in the center of Petri plate and the primed seeds with different concentrations of SA and H_2O_2 were placed at the 3 side of Petri plate. Seeds primed with sterilized distilled water for 5 minutes were also kept for comparison. A total of 36 Petri plates (3 replicates of each seeds and fungus) were kept at 30±2ºC for 5-7 days for detecting zone of inhibition of root infecting and germination of seeds.

Experimental site and setup: Experiment was conducted at the field of Department of Botany, University of Karachi, Pakistan during October to December 2017. The soil of the experimental field was sandy loam. Experiment was performed in randomized complete block design with three replication and eight treatments. Plot size was 1.2 m × 0.9 m. Cowpea and mung bean seeds were surface sterilized using sodium hypochlorite for three minutes (1.0 %) solution and then washed the residual chlorine with sterilized distilled water. Concentrations (0.5%) of SA and H_2O_2 were prepared and seeds soaked in priming solution for 5 minutes respectively at 30-32ºC. The primed seeds were then air dried at room temperature on sterilized filter paper to obtain its original moisture, after which, sown in plots (10 seeds/plot). Unprimed seed and seeds primed with sterilized distilled water were served as control. Plots were watered daily for sufficient amount of moisture upto yield. Plants were harvested for the observation of growth parameters like plant length, shoot weight, root length and weight, number of pods, length and weight of pods and number of nodules. Roots of each plant after harvesting washed with sterilized distilled water then sterilized with sodium hypochlorite (1.0%), transferred on Potato Dextrose Agar (PDA) Petri plates. These PDA poured plates containing antibiotics (penicillin and streptomycin) to avoid bacterial contamination and were incubated for 5-7 days at 30-32ºC for the isolation of root infecting fungi.

Measurement of chlorophyll a fluorescence: Chlorophyll a fluorescence was observed by dark adopted quantum using opti-sciences chlorophyll fluorometer where maximal quantum yield of PSII photochemistry (Fv/Fm) and performance index (PI_{ABS}) were measured (Strasser *et al.*, 2004).

Analysis: Obtained data were subjected to statistical analysis and least significant difference (LSD) test at $P<0.05$ and Tukey's HSD were performed to determine the significance difference between means.

Results

***In vitro* activity of SA and H_2O_2:** Chemical priming using SA and H_2O_2 with different concentrations (0.5, 1, 2%) showed prominent zones of inhibition when 2% SA and H_2O_2 (3, 3.2 mm) were used as mung bean seeds priming against *M. phaseolina*. However, in case of cowpea, priming with both chemicals at 2% concentration gave maximum zone against *R. solani*. *F. oxysporum* was also inhibited at 0.5, 1 and 2% concentrations when seeds of mung bean and cowpea were primed with SA and H_2O_2 (Tables 1, 2). It is interesting to note that 0.5% concentration of both chemicals promote germination in comparison to other concentrations of chemicals used. However, priming with water also revealed seed germination with little or no zone of inhibition against root infecting fungi (Tables 1, 2).

Effect on growth and root infecting fungi: Chemical priming with H_2O_2 and SA revealed enhancement in the growth of tested plants when applied to field at the rate of 0.5% (As in *In vitro* studies showed that 0.5% enhances germination compared to other concentrations so this concentration is selected for field trial). Cowpea primed seeds with H_2O_2 gave 100% germination. However, seed priming with SA seems to reduce the germination % as compared to control. Shoot length, shoot weight, root length, root weight, number of nodules, number, length and weight of pods were significantly ($p<0.05$) increased when cowpea seeds primed with H_2O_2 at 0.5 % as compared to SA primed seeds (Table 2). It was interesting to note that root infecting fungi like *M. phaseolina*, *R. solani* and *Fusarium* species were also decreased by application of H_2O_2 at 0.5% as compared to other treatments (SA and hydropriming). Seed priming with SA also showed effective and significant ($p<0.01$; 0.05) results in reduction of root infecting fungi (Table 3).

Table 1. *In vitro* studies showing antifungal activities of mung bean seeds priming against root infecting fungi.

Root infecting fungi	Zones of inhibition (mm)				
	Control	Water	Seeds priming with hydrogen peroxide		
	0%	0.0%	0.5%	1%	2%
Germination %	66.66	66.66	100	0.00	0.00
Fusarium oxysporum	1.2	1.5	1.8	2.3	2.5
R. solani	1.3	1.7	1.8	2.1	2.4
M. phaseolina	1.1	1.4	1.7	2.5	3
			Seeds priming with salicylic acid		
Germination %	66.66	66.66	100	33.33	0.00
Fusarium oxysporum	1.3	1.5	1.7	2.1	2.6
R. solani	1.4	1.5	1.8	2.2	2.4
M. phaseolina	1.2	1.4	1.9	2.3	3.2

Table 2. *In vitro* studies showing antifungal activities of cowpea seeds priming against root infecting fungi.

Root infecting fungi	Zones of inhibition (mm)				
	Control	Water	Seeds priming with hydrogen peroxide		
	0%	0.0%	0.5%	1%	2%
Germination %	33.33	66.66	100	0.00	0.00
Fusarium oxysporum	1.1	1.4	1.6	2.2	2.5
R. solani	1.4	1.5	1.8	2.3	3.1
M. phaseolina	1.5	1.5	1.9	2.6	2.7
			Seeds priming with salicylic acid		
Germination %	66.66	66.66	100	0.00	0.00
Fusarium oxysporum	1.2	1.5	1.8	1.9	2.1
R. solani	1.6	1.7	2.1	2.6	3
M. phaseolina	1.7	1.7	1.9	2.8	2.4

Similar to cowpea primed seeds, mung bean seeds after priming with H_2O_2 at 0.5% gave significant increased shoot length, shoot weight, root length, root weight, germination %, number of nodules, number, length and weight of pods ($P<0.05$, 0.01). However, reduction in colonization of *Fusarium* species on mung bean roots was accompanied by priming with SA while *M. phaseolina* and *R. solani* colonization was decreased by seeds priming with H_2O_2 followed by SA at 0.5% ($p<0.05$; Table 4).

Effect on photosynthesis: Data obtained for Fv/Fm was drastically increased in the cowpea seeds primed with water followed by SA and H_2O_2 (Fig. 1). Moreover, increased performance index (PI) was recorded in cowpea seeds primed with H_2O_2 while seeds primed with water showed decreased performance index. Further, water primed seeds of mung bean showed increased Fv/Fm followed by SA while maximum PI was obtained when mung bean seeds treated with H_2O_2. However, SA primed mung bean seeds showed decreased in PI (Fig. 2).

Discussion

As efficient seed germination and establishment of early seedlings are essential components for production of crops under stressful environmental conditions (Chen & Arora, 2013). Seed priming is said to be a controlled hydrated process which improves the metabolic activities of germination with prevention of radical protrusion leading an increment in germination with faster and synchronous emergence at suboptimal temperature (Carvalho *et al*., 2011). Priming of seed not only induced germination but also enhances seedling growth with stimulation of antioxidant activities in different crops (Afzal *et al*., 2012a; Afzal *et al*., 2012b; Bailly, 2004). Present study showed that both chemicals used for priming increased growth of plant with an effective reduction of root infecting fungi. However, priming with H_2O_2 showed more pronounced results in increment of shoot length, root length, shoot weight, root weight, number of nodules, number, length and weight of pods of both cowpea and mung bean seeds. Initially, H_2O_2 was recognized as toxic for plant cell causing various damages in cell organization and in losing cell viability. The important role of H_2O_2 as a signaling molecule in plants has been described from 1990s. Hydrogen peroxide has generally viewed as signal molecule in seed physiology which triggers acclimation in many species under abiotic and biotic conditions and plays an important role in regulation of seed germination (Wang *et al*., 2016; Paparella *et al*., 2015). When ascorbic acid, salicylic acid and hydrogen peroxide used for pretreatment of maize seeds, interestingly hydrogen peroxide gave more beneficial result compared to salicylic acid and ascorbic acid (Ahmed *et al*., 2015). This finding clearly showed that H_2O_2 was effectively imbibed by the seeds, sensed rapidly and transported to the seedlings of both crops (cowpea and mung bean). This accelerated growth was responsible for faster nutrient uptake, resulting in better production of crop (Revilla *et al*., 1999).

Table 3. Chemical priming of cowpea seeds with H_2O_2 and SA on the growth traits and on root rot fungi under field condition.

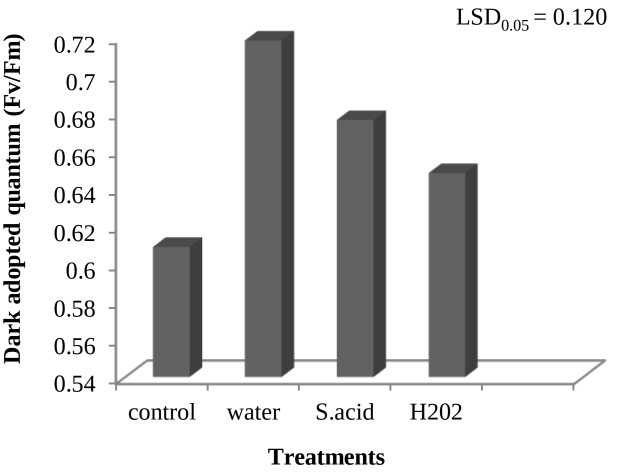
Priming treatments	Growth traits									Colonization % of root rot fungi		
	Germination %	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. of pods	Pod length	Pod weight	No. of nodules	*Fusarium* spp.	*R. solani*	*M. phaseolina*
Unprimed	93.33a	33.30a	2.14a	13.44a	1.59a	0a	0a	0a	7a	70.07a	46.66b	66.66b
Hydropriming	93.33a	33.84a	2.15a	14.2ab	1.91ab	2a	3.37a	1.13a	5a	40a	26.66a	40ab
H_2O_2	100a	36.36b	2.46a	15.11b	2.02b	4a	5.45a	1.80a	8a	40a	26.66a	20a
SA	80a	34.11a	2.2a	14.26ab	1.83ab	2a	3.28a	1.37a	7a	53.33a	33.33ab	46.66ab
$LSD_{0.05}$	19.76	1.565	0.375	1.015	0.242	3.86	4.230	1.531	3.562	25.49	14.380	20.33

Treatment bars with same letters are not significantly different from each other according to Tukey's HSD.

Table 4. Chemical priming of mung bean seeds with H_2O_2 and SA on the growth traits and on root rot fungi under field condition.

Priming treatments	Growth parameters									Colonization % of root rot fungi		
	Germination %	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. of pods	Pod length	Pod weight	No. of nodules	*Fusarium* spp.	*R. solani*	*M. phaseolina*
Unprimed	100a	34.59a	2.18a	13.63a	1.73a	0a	0a	0a	6a	63.4a	46.66a	66.66c
Hydropriming	100a	33.65a	2.16a	13.25a	1.82a	4ab	3.7bc	1.36a	8a	33.33a	46.66a	33.33bc
H_2O_2	100a	36.31a	2.72a	15.66b	1.98a	7c	5.63c	1.89a	9a	40a	26.66a	20a
SA	93.33a	34.63a	2.19a	14.21ab	1.73a	6bc	5c	1.74a	6a	33.33a	40a	40bc
$LSD_{0.05}$	10.870	1.599	0.533	1.705	0.288	1.762	3.454	1.579	4.682	28.24	38.81	52.133

Treatment bars with same letters are not significantly different from each other according to Tukey's HSD.



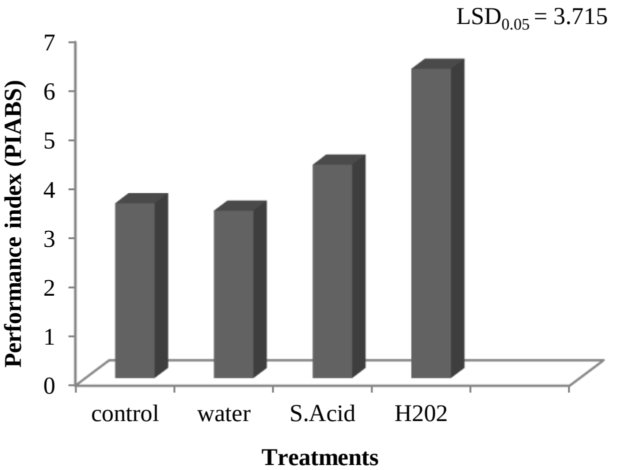
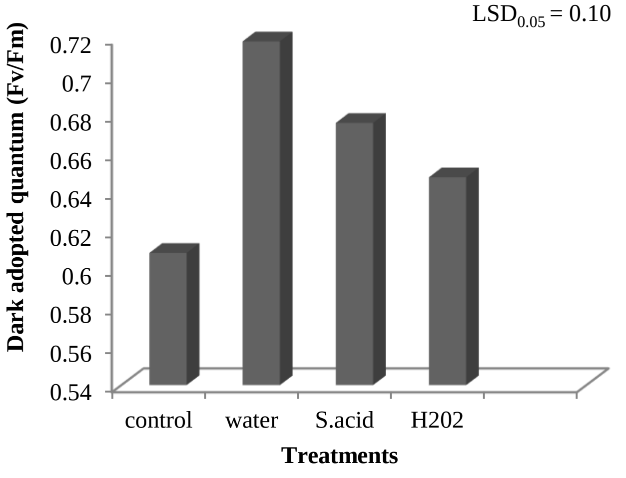


Fig. 1. Effect of chemical priming on Fv/Fm and Performance index of cowpea plants.

H_2O_2 has also the ability to protect against pathogens. This hypothesis is based on the role of oxidative burst during pathogen infection leading to induction of programmed cell death as it contains antimicrobial properties (Coll *et al*., 2011). Our findings clearly correlate with above hypothesis where *Fusarium* spp., *M. phaseolina* and *R. solani* were reduced with the application of H_2O_2 as compared to SA at 0.5% in cowpea and mung bean seeds except of *Fusarium* spp., which was reduced due to priming with SA in mung bean seeds. Various evidences present which showed role of H_2O_2 in protecting against pathogens during germination. During seedling development of *Lupinus luteus Fusarium oxysporum* was inoculated on embryonic axes causing the accumulation of H_2O_2 and free radicals (Morkunas *et al*., 2004). Nandi *et al*., (2017) observed the mycelial growth of *F. moniliformae* was inhibited by the use of 2% H_2O_2. According to the statement of Szopinska (2014), hydrogen peroxide treatment regardless of concentration improved the health of seeds with reduction in fungi percentage.

Physiological status particularly alterations of Chlorophyll-a fluorescence is nowadays widely applied technique for assessing performance of plants. When dark adopted leaves is exposed to short light pulse with high PPFD, fm (maximal level of fluorescence) is generated. Fv/Fm ratio gave an estimate of the maximum quantum efficiency of PSII photochemistry which is widely used to detect stress induced perturbations in the photosynthesic apparatus (Butler, 1978). Present result observed an increased Fv/Fm value in the cowpea seeds primed with water followed by SA and H_2O_2 while H_2O_2 primed cowpea bean seeds showed decreased PI. Development of slowly relaxing quenching processes and photodamage due to PSII reaction are responsible in reduction of maximum quantum efficiency of PSII (Neil & Rosenqvist, 2004). Uchida *et al*., (2002) and Wahid *et al*., (2007) observed that rice and wheat plants at low levels of hydrogen peroxide improved the growth and physiological performances that enhanced capacity of withstand in high salinity conditions due to prevention of oxidative damage and also increased plant growth.

In conclusion, seed priming was helpful method in order to improve germination, seedling growth and tolerance level of plant against root infecting fungi present in soil. Seed priming using 0.5 % H_2O_2 in field was more effective in enhancing growth attributes as compared to SA and hydropriming. The 0.5% concentration of H_2O_2 and SA seems to be suitable concentration to be used in field and generate new perspectives that will give more comprehensive explanation of processes for further studies.



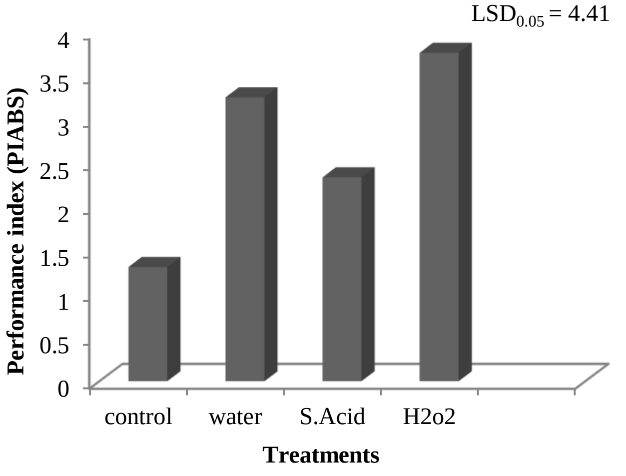


Fig. 2. Effect of chemical priming on Fv/Fm and Performance index of mung bean plants.
S. Acid = Salicylic acid; H_2O_2 = Hydrogen peroxide; Fv/Fm = Maximum quantum yield

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