Growth, photosynthesis, and antioxidant responses of *Vigna unguiculata* L. treated with hydrogen peroxide

Syed Aiman Hasan, Mohd. Irfan, Y.S. Masrahi, Mohamed Asmaa Khalaf and Shamsul Hayat

*Cogent Food & Agriculture* (2016), 2: 1155331
Growth, photosynthesis, and antioxidant responses of *Vigna unguiculata* L. treated with hydrogen peroxide

Syed Aiman Hasan¹, Mohd. Irfan*, Y.S. Masrahi³, Mohamed Asmaa Khalaf½ and Shamsul Hayat²

**Abstract:** Cowpea (*Vigna unguiculata* L.) is an important legume well grown in semi-arid and arid environment. Hydrogen peroxide solutions (0.1, 0.5, 1.0, and 1.5 mM) have been used to optimize growth and photosynthetic performance of cowpea plant at two growth stages [30 and 45 DAS (days of sowing)]. Foliar application of H₂O₂ at 0.5 > 1.0 mM solution at 29 DAS optimally promoted the photosynthetic attributes [leaf chlorophyll content, net photosynthetic rate (PN), water use efficiency, and maximum quantum yield of PSII (Fv/Fm)] and growth performance [root and shoot length; fresh and dry weight] of plants where the responses were more significant at the later growth stage. It was favored by activity of enzymes as carbonic anhydrase [CA; E.C. 4.2.1.1] and nitrate reductase [NR, E.C. 1.6.6.1] and those of antioxidant enzymes viz. peroxidase [POX; EC 1.11.1.7], catalase [CAT; EC 1.11.1.6], and superoxide dismutase [SOD; EC 1.15.1.1] and leaf proline content. Strengthened root system and antioxidant activity, particularly leaf proline level appeared to be the key factor for efficient photosynthesis and growth responses.

**Subjects:** Agriculture & Environmental Sciences; Botany; Nutrition

**Keywords:** antioxidant activity; growth; hydrogen peroxide; photosynthesis; *Vigna unguiculata*

**ABOUT THE AUTHOR**

Dr Mohd. Irfan obtained his doctoral degree from Department of Botany, Aligarh Muslim University, India. His research group at Aligarh, India, is working on plant stress physiological responses of crop plants and their amelioration using different plant growth regulators including recently recognized phytohormones. The thrust area of Dr Irfan is individual and interactive effects of heavy metals, plant growth regulators, and soil microbes in crop plants.

**PUBLIC INTEREST STATEMENT**

*Vigna unguiculata* or cowpeas are widely grown legume in arid and semiarid regions with good heat and drought resistance, therefore, is used as food vegetable, fodder, and in improving soil fertility. The legumes and seeds have high nutritive value and palatability. The performance of crop though varies depending upon edaphic and climatic conditions, genotype performance manifested as physiological outcome. Plant growth regulators are chemical signals which regulate plant growth and development under changing regimes of edapho-climatic conditions to unleash genotypic potential to its optimum. Hydrogen peroxide (H₂O₂), a well known secondary signal molecule has its dual effects depending upon its internal tissue level or externally applied concentration of H₂O₂. The effective dose of this plant growth regulator was tested as foliar spray for the cowpea crop in terms of growth, photosynthesis, enzymes activity of carbonic anhydrase, nitrate reductase, and those of antioxidant system at two stages of growth.
1. Introduction
Legumes are the members of family Leguminaceae, which are important grain yielding plants after Gramineae. Besides most important staple food crop worldwide, legumes are ecologically important due to their ability to fix nitrogen in soil. Herbaceous legume crops are important source of nitrogen-rich vegetables and pulses in diet. Cowpea (*Vigna unguiculata* L.; Walp) is an important leafy summer vegetable used for both grains and leaves. It is tolerant to drought and heat conditions, therefore are successful under arid and semiarid conditions. However, grains production of legumes, including cowpeas are greatly sensitized by the native abiotic stresses including heavy metal stress, salinity, and high temperature, registering yield output below the genetic potential of the plants.

Phytohormones are important regulators of plant growth both under non-stress and stressed conditions to unleash the genetic potential of plants in terms of yield output. Among non-classical plant growth regulators several natural molecules have been discovered amongst them hydrogen peroxide is important (Li, Qiu, Zhang, & Wang, 2011; Zelinová, Bočová, Huttová, Mistrik, & Tamás, 2013). It counteract biotic stress (Małolepsza & Różalska, 2005; Orozco-Cardenas, Narváez-Vásquez, & Ryan, 2001) re-establishing the cellular redox status (Kumar, Goswami, Singh, Rai, & Rai, 2013) for growth and development. It interact with certain other plant hormones as abscissic acid, nitric oxide, brassinosteroids, etc. (Jiang et al., 2012; Kumar, Sirhindi, Bhardwaj, Kumar, & Jain, 2010; Liao, Huang, Yu, & Zhang, 2012; Małolepsza & Różalska, 2005). Since the positive roles of reactive oxygen species have been recognized in normal plant growth and metabolism, importance of H$_2$O$_2$ emerged in different plant developmental aspects. As the dose-dependent duality of H$_2$O$_2$ was accepted, species and genotypic responses of it were tested under abiotic stress and normal plant growth conditions. The external application of hydrogen peroxide has been proved promising in improving the growth responses of several plants including legumes, modulating the physiological and biochemical responses (Ashfaq, Khan, & Khan, 2014; Deng et al., 2012; Ishibashi et al., 2011). Since a threshold level of reactive oxygen species is important to trigger the plant growth (Dat et al., 2000), hydrogen peroxide is recognized as a versatile molecule in this network (İşeri, Körpe, Sahin, & Haberal, 2013; Quan, Zhang, Shi, & Li, 2008).

Plants never grow in optimal growth conditions and face a range of diurnal changing environments of water, heat, radiations, and edaphic alterations to manifest suboptimal growth of their genetic potential. An optimal concentration of plant growth regulator, based on age and mode of treatment may unleash this genetic potential to provide better outcome. The role of H$_2$O$_2$ as a secondary messenger is well established in the literature (Neill, Desikan, Clarke, Hurst, & Hancock, 2002; Orozco-Cardenas et al., 2001; Quan et al., 2008; Veal, Day, & Morgan, 2007) and it has been reported to have roles in physiological regulation under abiotic and biotic stress conditions (Małolepsza & Różalska, 2005; Orozco-Cardenas et al., 2001). Application of H$_2$O$_2$ has also been shown to improve the plant growth metabolism and physiological performance in various crop plants (Ishibashi et al., 2011; Kao, 2014) in concentration-dependent manner (Razem, 2008).

The present study was carried out to evaluate the potential of plant growth regulators, hydrogen peroxide, against physiological responses in cowpea emphasizing on growth, photosynthesis, and antioxidant system activity. Cowpea (*Vigna unguiculata* L.) has been tested against their different concentrations sprayed on leaves at 30 days of sowing (DAS). The aim was to test the comparative growth responses in correlation with photosynthetic attributes and activity of antioxidant system in *V. unguiculata* treated with H$_2$O$_2$.

2. Materials and methods

2.1. Preparation of plant growth regulators
Hydrogen peroxide (H$_2$O$_2$) was obtained from Sigma Chemicals, USA. Molar stock solution of H$_2$O$_2$ was prepared by dissolving required quantity of double distilled water (DDW) in 100 cm$^3$ volumetric flasks. The desired concentrations of hydrogen peroxide (0, 0.1, 0.5, 1.0, and 1.5 mM H$_2$O$_2$ solutions) were prepared by the dilution of stock solution. Five cubic centimeter surfactant “Tween-20” was added to the solution using DDW at the time of spray.
2.2. Plant material and experimental setup
Healthy, uniform seeds of *Vigna unguiculata* L. Walp were obtained from authentic source. Surface-sterilized seeds (with 0.01% HgCl₂ solution for 2 min) were washed repeatedly with DDW to remove adhering particles of HgCl₂. The experiment was arranged in a completely randomized block design in the natural environment. The experiment was set up in late February of 2014–2015, under ambient environmental conditions with optimum temperature varied from 10 to 30°C.

2.2.1. Soil characteristics
Seeds were sown in earthen pots (25 × 25 cm) filled with garden soil and farmyard manure in ratio 6:1 v/v. The soil samples were analyzed in the Soil Testing Laboratory, Government Agriculture Farm, Quarsi, Aligarh. The physical–chemical properties of soil included; sandy loam in texture, pH 7.94, E.C. (1:2) 0.48 mhos cm⁻¹, organic carbon 1.22%, available N; 123.36–134.2 kg/ha (low), P; 31.67–35.46 kg/ha (low) and K; 118.64–122.52 kg/ha (medium), respectively. The farmyard manure was prepared using cow dung and vegetable domestic waste mixture when completely decomposed. The soil was amended with uniform recommended basal dose of N, P, and K from urea, single super-phosphate and muriate of potash added at 40, 138, and 26 mg kg⁻¹ of soil, respectively. The seeds of cowpea were sown at the rate of eight seeds per pot. After one week, plants were thinned to three plants per pot. The foliage of plants were sprayed with hydrogen peroxide (0, 0.1, 0.5, 1.0, or 1.5 mM H₂O₂) at 29 DAS and sampled at 30 and 45 DAS of vegetative stage for various parameters.

2.3. Growth analysis of plants
The plants were gently dig from the pots along with adhered soil and dipped in a bucket filled with tap water. The soil particles were gently removed from plant roots. The length and fresh mass of shoot and root separately were measured using a meter scale and squares covered by the three fully developed upper leaves were counted on graph paper for average leaf area. The plant parts were then placed in an oven at 80°C for 72 h. The dried shoot and root were then weighed to record dry mass.

2.4. Leaf pigment content and photosynthetic parameters
The leaf chlorophyll and carotenoid content was measured following the method of Mackinney (1941). Photosynthetic parameters [net photosynthetic rate (PN), water use efficiency (WUE), and maximum quantum yield of PSII (Fv/Fm)] were measured in a well-expanded upper third leaf while attached to the plant using an infrared gas analyzer portable photosynthetic system (Li-COR 6400, Li-COR, Lincoln, NE, USA), between 11:00 and 12:00 h under clear sunlight. The atmospheric conditions during measurement were photosynthetically active radiation, 1,016 ± 6 μmol m⁻²s⁻¹, relative humidity 60 ± 3%, atmospheric temperature 22 ± 1°C, and atmospheric CO₂ 360 μmol mol⁻¹. The duration of the measurement of each sample was 10 min after the establishment of steady-state conditions inside the measurement chamber.

2.5. Carbonic anhydrase (CA) activity
The activity of carbonic anhydrase was determined by adopting the procedure described earlier by Dwivedi and Randhawa (1974). Fresh leaf samples (0.2 g) were cut into small pieces and suspended in 10 ml of 0.2 M cysteine hydrochloride solution. The samples were incubated at 4°C for 20 min. The leaf pieces were blotted dry and transferred to test tubes containing 4 ml of phosphate buffer (pH 6.8) followed by the addition of 4 ml of 0.2 M alkaline bicarbonate solution and 0.2 ml of 0.002% bromothymol blue indicator. The test tubes were incubated at 4°C for 20 min. The reaction mixture was titrated against 0.05 N HCl, after the addition of 0.2 ml of methyl red indicator. The results are expressed as mol (CO₂) kg⁻¹ (leaf FM) s⁻¹.

2.6. Nitrate reductase (NR) activity
Nitrate reductase activity was measured by the method of Jaworski (1971) in fresh leaf samples that were cut into small pieces. The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5), KNO₃, and isopropanol that were incubated at 30°C for 2 h. After incubation, sulfanilamide and N-1-naphthylethylenediamine hydrochloride solutions were
added. The absorbance was read at 540 nm and the activity of NR [n mole NO₂ g⁻¹ (FM) s⁻¹] was calculated.

2.7. Antioxidant system activity
The activity of peroxidase (POX) and catalase (CAT) was assayed following the procedure described by Chance and Maehly (1955). The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971). The amount of enzyme which causes 50% inhibition in photochemical reduction of NBT was considered as one enzyme unit. The proline content in fresh leaf samples was determined by the method of Bates, Waldren, and Teare (1973). The absorbance of the toluene layer was read at 528 nm, on a spectrophotometer (Milton & Roy, USA).

3. Statistical analysis
The experiment was conducted according to simple randomized block design. Each treatment was replicated five times and three plants per pot were maintained where each pot was considered as a replicate. Treatment means were compared by the analysis of variance using R ver. 3.1.0 for Windows (http://www.r-project.org/). Least Significant Difference between treatment means was calculated at 5% probability level (p < 0.05).

4. Results

4.1. Growth Parameters
All the growth values (leaf area per plant, length, fresh and dry mass of shoot and root) increased with the treatment, as compared to the water-treated (Control) plants at 45 DAS (Figures 1(a)–(g)). All the concentrations of H₂O₂ increased the above-mentioned growth parameters. However, the best concentration was recorded to be 0.5 mM H₂O₂ foliar spray which induced the growth more effectively than the other concentrations. This concentration registered percent increase in root and shoot lengths by 25 and 35%, for fresh masses by 39, 33%, and for dry masses by 20, 29%, respectively, compared to controls. The increase in leaf area was by 25% as compared to water-sprayed control plants.

4.2. Leaf pigment contents and photosynthetic parameters
The foliar treatment (H₂O₂; 0, 0.1, 0.5, 1.0, or 1.5 mM) significantly improved the pigment (total chlorophyll and carotenoid) content at later growth age (45 DAS) as compared to early growth stage (30 DAS), where 0.5 mM foliar spray of H₂O₂ induced the level of these pigments irrespective of growth stage (Figures 2(b) and (c)). The net photosynthetic rate (Pn) along with their attributes (WUE and Fv/Fm; maximum quantum yield of PSII) also increased as the growth progressed from 30 to 45 DAS, as compared to the control plants (Figures 2(a), (d) and (e)). Leaves of V. unguiculata sprayed with 0.5 mM H₂O₂ showed best improvement in chlorophyll and carotenoid level 28 and 39% (30 DAS) and by 34% (45 DAS), while Pn, WUE, and Fv/Fm increased by 38, 36, and 26 and 45%, 32, and 20%, at 30 and 45 DAS, respectively, as compared to their controls. However, the order of improvement of pigments level and photosynthetic attributes was 0.5 mM > 1.5 mM > 1 mM > 0.1 mM > 0.0 mM (control).

4.3. Activity of carbonic anhydrase (CA) and nitrate reductase (NR) enzyme
The activity of CA and NR both increased significantly with respect to foliar application of different molar solutions (0, 0.1, 0.5, 1.0, or 1.5 mM) H₂O₂ (Figures 2(f) and (g)). Out of the various concentrations of H₂O₂, the maximum activity was recorded in plants treated with 0.5 mM solution, whereas lowest activity was recorded in plants received 1.5 mM solution as compared to control. Both the enzymes reflected increasing values of enzymes activity as the growth progressed from 30 to 45 DAS. The percent increase in CA activity by different molar solutions of H₂O₂ was 27, 52, 36, and 23%, whereas in NR activity was 38, 52, 36, and 32%, at 45 DAS, respectively, compared to control plants. The increase in CA and NR activity was more at 30 DAS as compared to later growth stage (45 DAS).
4.4. Antioxidant system activity

The data depicted in Figures 3(a)–(d) clearly indicated an increase in the activity of all the antioxidant enzymes as growth progressed and also in response to different concentrations (H$_2$O$_2$; 0, 0.1, 0.5, 1.0, or 1.5 mM) of treatments. Among the four tested concentrations, 0.5 mM H$_2$O$_2$ maximum enhanced the activity of POX, CAT, and SOD. Higher activity was recorded at growth stage sampled after foliar treatment than latter growth stage. Percent increase of CAT, POX, and SOD was by 57, 27, and 38% at 30 DAS, while it increased by 29, 42, and 19% at 45 DAS, respectively, than the control plants. Also a significant increase in the leaf proline content in response to different concentrations H$_2$O$_2$ was recorded as compared to control. As the growth progressed, the proline content decreased similar to that of antioxidant enzymes.
5. Discussion
Most of the work on H$_2$O$_2$ application at low concentration treatments, studied in seed germination or seedling growth has given positive response. The experimental results suggested that H$_2$O$_2$ treatment increased the plant growth parameters (length, fresh and dry weight of shoot and root, leaf area) at 45 DAS when sprayed at 29 DAS on foliage. However, higher and lower concentration of H$_2$O$_2$ than the optimum level (here 0.5 mM) has not induced growth parameters optimally. Lower concentrations of H$_2$O$_2$ alone or with nitric oxide positively influence adventitious root growth of marigold and sweet potato seedlings (Deng et al., 2012; Liao, Xiao, & Zhang, 2009; Liao et al., 2012). A different set of plant hormones have been recorded to interact with H$_2$O$_2$ during germination and early plant growth (Barba-Espin et al., 2010) and under different set of growth conditions. H$_2$O$_2$-mediated auxin-induced gravitropic responses have also been worked out in maize roots (Joo, Bae, & Lee,
2001). Auxin-induced H$_2$O$_2$ concentration in tomato root tips inhibits root growth (Ivanchenko et al., 2013). The increased shoot length was also calculated in this experiment (Figures 1(b) and (c)) which might be due to auxin-directed H$_2$O$_2$ signaling (Joo et al., 2001). H$_2$O$_2$ induced the germination and early seedling growth of barley and wheat (Çavuşoğlu & Kabar, 2010; Hameed, Farooq, Iqbol, & Arshad, 2004), probably counteracting the ABA action. The growth of plant and plant organs depends upon the induction of water and mineral uptake through root hair plasma membranes, regulated by the hormonal network to reset the growth redox in meristematic tissues. Qiu, Li, Bi, and Yue (2011) suggested that H$_2$O$_2$ metabolism in wheat seedling was involved as a signal in the processes of laser-induced water acclimation, the osmo-protective effect they related with NADPH oxidase-dependent H$_2$O$_2$ production. The fresh and dry weight of the plants in our experiment (Figures 1(d) and (f)) also increased with H$_2$O$_2$ treatment. Increased adventitious root growth and absorption of mineral (especially P) ions favor root nodule formation (D’Haeze et al., 2003). A positive role of SODs is tested by Rubio et al. (2004) as a source of H$_2$O$_2$ in indeterminate alfalfa (Medicago sativa) and pea (Pisum sativum) nodules where abundant H$_2$O$_2$ reflected the oxidative stress inducing nodule senescence which was associated with the degrading bacteroids in the senescent zone. The leaves of soybean foliar pre-treated sprayed with H$_2$O$_2$ under drought stress had higher relative water content than non-treated soybean leaves (Ishibashi et al., 2011). Treatment with H$_2$O$_2$ or salicylic acid caused significant increase in growth parameters in tomato cultivars, which was due to marked increase in endogenous growth regulators as GA$_3$, IAA, and ABA (Orabi, Dawood, & Salman, 2015). Exogenous H$_2$O$_2$ application to legumes also increased the dry matter production (Figures 1(e) and (g)). Increased plant dry matter was also recorded in the leaves of wax apple (Khandaker, Boyce, Osman, & Hossain, 2012), maize plants with induced mineral content and level of osmotic solutes (Guzel & Terzi, 2013; Terzi, Kadioglu, Kalaycioglu, & Saglam, 2015) by exogenous H$_2$O$_2$ treatment. Generation of H$_2$O$_2$ in the cell wall apoplastic space is reported and has been shown to be required for the formation of cell cross-linking of wall polymers (Eistner & Heupel, 1976; Mader, Ungemach, & Schloss, 1980). The increased leaf area in tested plants (Figure1(a)) appears due to induction of cell proliferation. In multicellular organisms, H$_2$O$_2$ can also activate signaling pathways to stimulate cell proliferation (Foreman et al., 2003; Geiszt & Leto, 2004), differentiation (Konieczny, Banaś, Surówka, Michalec, & Miszalski, 2014; Potikha, Collins, Johnson, Delmer, & Levine, 1999), and seedlings elongations.
Hasan et al., Cogent Food & Agriculture (2016), 2: 1155331
http://dx.doi.org/10.1080/23311932.2016.1155331

(Barba-Espin et al., 2010). Similar result in mung bean was also obtained by Fariduddin, Khan, and Yusuf (2014).

Present study indicated that the treatment of H$_2$O$_2$ significantly increased the pigment levels and photosynthetic attributes i.e. net photosynthetic rate, WSE, and maximum quantum yield of PS II, in the cowpeas (Figures 2(a)–(e)). H$_2$O$_2$ treatment with BRs caused significant increases in chlorophyll level in *Vigna radiata* (Fariduddin et al., 2014) and in sand-cultured tomato cultivars (Orabi et al., 2015), which was suggested due to marked increase in endogenous growth regulators. Formation of H$_2$O$_2$, can also take place through thermal dissipation from antenna inducing excess electron leakage from photosynthetic electron transport chain, to molecular oxygen (Mehler reaction). It was indicated that H$_2$O$_2$, can diffuse through the chloroplast envelope aquaporins, where CA presumably remains attached (Borisova et al., 2012). Activity of CA with H$_2$O$_2$-mediated increased influx of stomatal CO$_2$ can cooperatively induce the net photosynthetic rate as was reported in *Vicia faba* stomatal guard cells (Jannat et al., 2011; Zhang et al., 2001) and maize plants (Gondim et al., 2013). A significantly increased quantum efficiency of PSII (Fv/Fm value) was obtained with the lower concentration (0.1, 0.5, and 1.0 mM) application of H$_2$O$_2$, as observed in the experiment. The effect of H$_2$O$_2$ on Fv/Fm might be due to interaction with ABA and NO mediated which was also shown by (Neill, 2007; Neill et al., 2002; Yang, Yun, Zhang, & Zhao, 2006). Hydrogen peroxide works as a secondary messenger for Brassinosteroids induced CO$_2$ assimilation and carbohydrate metabolism (Jiang et al., 2012). It was also recorded that exogenous H$_2$O$_2$ increased photosynthetic rates in the leaves of wax apple under field conditions (Khandaker et al., 2012).

Often elevated level of H$_2$O$_2$, is correlated with oxidative stress which resulted into increased level of antioxidant molecules including carotenoids such as beta carotene and xanthophyll (Upadhyaya, Khan, & Panda, 2007). The carotenoid endoperoxide produced from a reaction between β-carotene and reactive oxygen species Ramel, Mialoundama, and Havaux (2012). Carotenoids besides working as antenna are important antioxidants in photosynthetic systems (Larson, 1988) absorbing short wavelength energy. Increased carotenoid level concomitant with carotenoid level is often correlated with increasing age or stress (Prochazkova, Sairam, Srivastava, & Singh, 2001). Cowpea Plants sprayed with chitosan reflected increased accumulation of H$_2$O$_2$, concomitantly increased the carotenoid level in leaves (Farouk, Ramadan, & Showler, 2013).

As it is clear from the results, application of H$_2$O$_2$, significantly induced the activity of two enzymes i.e. carbonic anhydrase and nitrate reductase (Figures 1(f)–(g)). Among the different concentrations of H$_2$O$_2$, solutions (0, 0.1, 0.5, 1.0, 1.5 mM) used as foliar spray, 0.5 mM best optimized the enzyme’s activity which could be due multiple factors viz. increased substrate availability, redox regulation of enzymes transcripts and their translation to increase their cellular pool, and availability of co-factors. Hydrogen peroxide (H$_2$O$_2$) regulated increased adventitious rooting suggesting increased surface area of absorption for important critical mineral ions including N, P, and K. Phosphorus is known to be required for the formation of root nodules (Tang, Hinsinger, Drevon, & Illard, 2001) which further increase the N assimilation from root hairs (Fujikake et al., 2003). Increased substrate (nitrate) availability could possibly upregulate the activity of NR in legume leaves. Carbonic anhydrase activity is regulated by the availability of cellular availability of CO$_2$, taken up through stomatal activity. H$_2$O$_2$ regulates the stomatal conductance and hence the CO$_2$ exchange (Gondim, Miranda, Gomes-Filho, & Prisco, 2013; Jannat et al., 2011; Zhang et al., 2001). Here, again increased availability could positively regulate the activity of CA in photosynthesizing leaves. The enzyme CA has also been detected near the thylakoid aquaporins, and was also suggested earlier to have role in the regulation of photosynthetic electron transport chain (Borisova et al., 2012; Stemler, 1997). Root absorption of mineral ions (e.g. Zn, Co, Fe, etc.) to work as co-factors helping to catalyze the activity of CA and NR may favorably be suggested for the increased activity of these enzymes by H$_2$O$_2$, foliar treatment.

Hydrogen peroxide (H$_2$O$_2$) has a central role in growth and development signal-information processing (Petrov & Breusegem, 2012). H$_2$O$_2$, is also perceived as stress signal (Hung, Yu, & Lin, 2005) with its role in multiple stress through alteration of antioxidant activity (Abass & Mohamed, 2011;
Gao, Guo, Lin, Fang, & Bai, 2010; Gondim et al., 2013; Guzel & Terzi, 2013; Hung, Wang, Ivanov, Alexieva, & Yu, 2007; Khan, Syeed, Masood, Nazar, & Iqbal, 2010; Kumar et al., 2010; Moskova, Todorova, Alexieva, & Segiev, 2014; Orabi et al., 2015; Zhang, Jia, Yu, Gao, & Bai, 2011). The H_2O_2 mediates the stress and defense response controlling plant cell signaling and gene expression patterns (George, 2014). The evidence of H_2O_2 signal crosstalk has also been marked with some very active species such as salicylic acid (Rao, Paliyath, Ormrod, Murr, & Watkins, 1997; Scott, Dat, Lopez-Delgado, & Foyer, 1999), ABA (Jannat et al., 2011; Vandenabeele et al., 2003), singlet oxygen (Laloi et al., 2007), brassinosteroids (Jiang et al., 2012), and nitric oxides (Neill et al., 2002; Tanou et al., 2010). The activity of antioxidant enzymes as CAT, POX, and SOD increased in the leaves which received the H_2O_2 as foliar spray (Figures 3(b), (c) and (d)). As H_2O_2 is the substrate of CAT and POX, the induction of its activity is but obvious. NADPH oxidase at apoplastic membranes mediates the self-propagation of H_2O_2 under the regulation of BRs. NADPH oxidase is an important enzyme participates in the formation of singlet oxygen or superoxides. Auxin, which directs the BRs signaling most often, has also been suggested to induce H_2O_2 level in meristems which signals to set redox status in favor of plant growth (Ivanchenko et al., 2013; Veal et al., 2007). H_2O_2 play a key role in indeterminate nodules formation in alfalfa (Medicago sativa) and pea (Pisum sativum) and nodule senescence (Rubio et al., 2004).

The level of proline increased in the plants exogenously sprayed with H_2O_2 on legumes foliage (Figure 1(a)). The increased relative water content due to H_2O_2 application (as discussed above) was positively correlated with the induced level of osmolites such as sugars, polyamines, and proline has been recorded in plant tissues which creates a negative potential for the absorption of water. Increased proline level was also recorded in several plants against H_2O_2 signaling (Fariduddin et al., 2014; Guzel & Terzi, 2013; Jiang et al., 2012; Moskova et al., 2014).

6. Conclusion
The positive role of H_2O_2 on plant growth and development is concentration and age dependent to promote its root system, photosynthesis and redox status for better growth and physiology. In cowpeas, 0.5 mM concentration optimally produced most of the growth and physiological features of plant favorably at 45 days after sowing (DAS) of plants when sprayed with H_2O_2 at 29 DAS on leaves.

Acknowledgment
Author Syed Aiman Hasan thankfully acknowledge project grant provided by Deanship of Scientific Research Center Jizan University, Jazan Kingdom of Saudi Arabia.

Funding
The authors received no direct funding for this research.

Competing interests
The authors declare no competing interest.

Author details
Syed Aiman Hasan¹
E-mail: aiman.uno@gmail.com
Mohd. Irfan²
E-mail: mohdirfan1983@gmail.com
Y.S. Masrahi³
E-mail: ymasrahi@gmail.com
Mohamed Asmaa Khalaf⁴
E-mail: akdem40@gmail.com
Shamsul Hayat⁵
E-mail: hayat_68@yahoo.co.in

¹ Department of Biology, College of Science for Girls, Jazan University, Jizan, P.O. Box 2079, Saudi Arabia.
² Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202002, India.
³ Faculty of Science, Department of Biology, Jazan University, Jazan, P.O. Box 2079, Saudi Arabia.

Citation information
Cite this article as: Growth, photosynthesis, and antioxidant responses of Vigna unguiculata L. treated with hydrogen peroxide, Syed Aiman Hasan, Mohd. Irfan, Y.S. Masrahi, Mohamed Asmaa Khalaf & Shamsul Hayat, Cogent Food & Agriculture (2016), 2: 1155331.

Cover image
Source: Authors.

References
Abass, S. M., & Mohamed, H. I. (2011). Alleviation of adverse effects of drought stress on common bean (Phaseolus vulgaris L.) by exogenous application of hydrogen peroxide. Bangladesh Journal of Botany, 41, 75–83.
Ashfaqe, F., Khan, M. I. R., & Khan, N. A. (2014). Exogenously applied H_2O_2 promotes proline accumulation, water relations, photosynthetic efficiency and growth of wheat (Triticum aestivum L.) under salt stress. Annual Research & Review in Biology, 4, 105–120.
Barba-Espin, G., Diaz-Vivancos, P., Clemente-Moreno, M. J., Albicete, A., Faize, L., Faize, M., ... Hernández, J. A. (2010). Interaction between hydrogen peroxide and plant hormones during germination and the early growth of pea seedlings. Plant Cell and Environment, 33, 981–994. http://dx.doi.org/10.1111/j.1365-3040.2010.01978.x
Botes, L. S., Wolden, R. P., & Yeare, I. W. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39, 205–207. http://dx.doi.org/10.1007/BF00018060
Beauchamp, C. O., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44, 276–287. http://dx.doi.org/10.1016/0003-2697(71)90370-8

Borisova, M. M., Kozulev, M. A., Rudenko, N. N., Noydon, I. A., Klunina, I. B., & Ivanov, B. N. (2012). Photosynthetic electron flow to oxygen and diffusion of hydrogen peroxide through the chloroplast envelope via aquaporins. Biochimica et Biophysica Acta (BBBA) - Bioenergetics, 1817, 1314–1321. http://dx.doi.org/10.1016/j.bbabio.2012.02.036

Čavuşoğlu, K., & Kabar, K. (2010). Effects of hydrogen peroxide on the germination and early seedling growth of barley under NaCl and high temperature stresses. Eurasian Journal of Biosciences, 4, 70–79. http://dx.doi.org/10.5053/ejbios

Chance, B., & Maehly, A. C. (1955). Assay of catalase and peroxidases. Methods in Enzymology, 2, 764–775. http://dx.doi.org/10.1016/0076-6879(55)50230-0

D’Haeze, W., De Rycke, R., Mathis, R., Goormachtig, S., Pagnotta, S., Verplancke, C., … Holsters, M. (2003). Reactive oxygen species during plant stress responses. Theoretical and Experimental Plant Physiology, 25, 251–260. http://dx.doi.org/10.1590/S1983-87402003000400003

Geitz, M., & Leito, T. L. (2004). The Nox family of NADPH oxidases: Host defense and beyond. Journal of Biological Chemistry, 279, 51715–51718. http://dx.doi.org/10.1074/jbc.M4000024200

George, M. P. (2012). The role of hydrogen peroxide in controlling plant cell signaling and gene expression patterns related to stress and defense responses. Student Pulse, 6(6), 1–3.

Gondhir, F. A., Miranda, R. D., Gomes-Filho, E., & Prisco, J. T. (2013). Enhanced salt tolerance in maize plants induced by H2O2, leaf spraying is associated with improved gas exchange rather than with non-enzymatic antioxidant system. Theoretical and Experimental Plant Physiology, 25, 251–260. http://dx.doi.org/10.1590/S1983-87402003000400003

Guzel, S., & Terzi, R. (2013). Exogenous hydrogen peroxide increases dry matter production, mineral content and level of osmotic solutes in young maize leaves and alleviates deleterious effects of copper stress. Botanical Studies, 54, 26. http://dx.doi.org/10.1007/s11738-019-9251-5

Hameed, A., Farooq, S., Iqbal, N., & Arshad, R. (2004). Influence of exogenous application of hydrogen peroxide on root and shoot growth of wheat (Triticum aestivum L.). International Journal of Agricultural Biology, 6, 366–369.

Hung, S. H., Yu, C. W., & Lin, C. H. (2003). Hydrogen peroxide functions as a stress signal in plants. Botanical Bulletin of Academia Sinica, 41, 1–10.

Hung, S. H., Wang, C. C., Ivanov, S. V., Alexieva, V., & Yu, C. W. (2000). Repetition of hydrogen peroxide treatment induces a chilling tolerance comparable to cold acclimation in mung bean. Journal of American Society for Horticultural Sciences, 132, 700–776.

Işeri, Öziüm Darcansoy, Körpe, D. A., Sahin, F. I., & Haberal, M. (2013). Hydrogen peroxide pretreatment of roots enhanced oxidative stress response of tomato under cold stress. Acta Physiologiae Plantarum, 35, 1905–1913. http://dx.doi.org/10.1007/s11738-013-1228-7

Ishibashi, Y., Yamaguchi, H., Yuasa, T., Iwao-Inoue, M., Arima, S., & Zheng, S. H. (2011). Hydrogen peroxide spraying alleviates drought stress in soybean plants. Journal of Plant Physiology, 168, 1562–1567. http://dx.doi.org/10.1016/j.jph.2011.02.003

Ivanchenko, M. G., den Os, D. D., Monshausen, G. B., Dubrovsky, J. G., Bednarova, A., & Krishnan, N. (2013). Auxin increases the hydrogen peroxide (H2O2) concentration in tomato (Solanum lycopersicum) root tips while inhibiting root growth. Annals of Botany, 112, 1107–1116. http://dx.doi.org/10.1093/aob/mct181

Jannott, R., Uroji, M., Morofuji, I., Islam, M. M., Bloom, R. E., Nakamura, Y., … Murata, Y. (2011). Roles of intracellular hydrogen peroxide accumulation in abscisic acid signaling in Arabidopsis guard cells. Journal of Plant Physiology, 168, 1919–1926. http://dx.doi.org/10.1016/j.jplph.2011.05.006

Jaworski, E. G. (1971). Nitrate reductase assay in intact plant tissues. Biochemical and Biophysical Research Communications, 43, 1274–1279. http://dx.doi.org/10.1016/S0006-291X(71)80010-4

Jiang, Y. P., Cheng, F., Zhou, Y. H., Xia, X. J., Mao, W. H., Shi, K., … Yu, J. Q. (2012). Hydrogen peroxide functions as a secondary messenger for brassinosteroids-induced CO2 assimilation and carbohydrate metabolism in Cucumis sativus. Journal of Zhejiang University Science B, 8, 811–823. http://dx.doi.org/10.1016/j.jus.2011.01.030

Joo, J. H., Bae, Y. S., & Lee, J. S. (2001). Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiology, 126, 1055–1060. http://dx.doi.org/10.1104/pp.126.3.1055
Kao, C. H. (2016). Role of hydrogen peroxide in rice plants. Crop Environment & Bioinformatics, 11, 1–10.

Khan, N. A., Syeed, S., Masood, M., Nazar, R., & Iqbal, N. (2010). Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. International Journal of Plant Biology, 1, 1.

http://dx.doi.org/10.4081/ipb.2010.e1

Khandaker, M. M., Boyce, A. N., Osman, N., & Hassain, A. B. M. S. (2012). Physicochemical and phytochemical properties of wax apple (Syzygium samarangense [Blume] Merrill & L.M. Perry var. Jambu Modul) as affected by growth regulator application. The Scientific World.
doi:10.11020/2012/728613

Koniczyc, R., Banas, A. K., Suriwoka, E. S. Z., Michalec, Z., & Miszalski, Z. (2014). Pattern of antioxidative enzyme activities and hydrogen peroxide content during developmental stages of phloemogenesis from hypocotyl explants of Mesembryanthemum crystallinum L. Plant Cell Reports, 33, 165–177.

http://dx.doi.org/10.1007/s00299-013-1520-4

Kumar, M., Sirhindi, G., Bhardwaj, R., Kumar, S., & Jain, G. (2010). Effect of exogenous H2O2 on antioxidative enzymes of Brassica juncea L. seedlings in relation to 24-epibrassinolide under chilling stress. Journal of Plant Biology, 24-epibrassinolide under chilling stress. Journal of Plant Biology, 31, 1744–1751.

http://dx.doi.org/10.1007/s00299-013-1520-4

Kumar, R. R., Goswami, S., Singh, K., Rai, G. K., & Rai, R. D. (2010). Effect of exogenous H2O2 on antioxidant enzymes of wax apple (Syzygium samarangense [Blume] Merrill & L.M. Perry var. Jambu Modul) as affected by growth regulator application. The Scientific World.
doi:10.11020/2012/728613

Liao, W. B., Huang, G. B., Yu, J. H., & Zhang, M. L. (2012). Nitric oxide and hydrogen peroxide in adventitious root formation and cellular determination in plants. Phytochemistry, 88, 842–96.

http://dx.doi.org/10.1016/j.phytochem.2012.08.029

Liao, W., Xiao, H., & Zhang, M. (2009). Role of nitric oxide and hydrogen peroxide in adventitious root development of marigold. Acta Physiologiae Plantarum, 31, 1279–1289.

http://dx.doi.org/10.1007/s11738-009-0367-3

Liao, W. B., Huang, G. B., Yu, J. H., & Zhang, M. L. (2012). Nitric oxide and hydrogen peroxide alleviate drought stress in marigold explants and promote its adventitious root development. Plant Physiology and Biochemistry, 58, 6–15.

http://dx.doi.org/10.1016/j.plaphy.2012.03.012

Mackinney, G. (1943). Absorption of light by chlorophyll solutions. Journal of Biological Chemistry, 140, 315–322.

Malolepsza, U., & Rätzska, S. (2005). Nitric oxide and hydrogen peroxide in tomato resistance. Plant Physiology and Biochemistry, 43, 623–635.

http://dx.doi.org/10.1016/j.plaphy.2005.04.002

Mader, M., Ungemach, Jutta, & Schloss, P. (1988). The role of peroxidase isoenzyme groups of Nicotiana tabacum in hydrogen peroxide formation. Planta, 174, 467–470.

http://dx.doi.org/10.1007/BF00380189

Maksyova, I., Todorova, D., Alexieva, V., & Seglev, I. (2014). Protective effect of hydrogen peroxide against paraquat toxicity in young pea plants: Possible role of endogenous polyamines. American Journal of Plant Sciences, 5, 3408–3416.

http://dx.doi.org/10.4236/ajps.2014.522356

Nell, S. (2007). Interactions between abscisic acid, hydrogen peroxide and nitric oxide mediate survival responses during water stress. New Phytologist, 175, 4–6.

http://dx.doi.org/10.1111/nph.2007.175.issue-1

Nell, S. J., Desikan, R., Cirkle, A., Hurst, R. D., & Hancock, J. T. (2002). Hydrogen peroxide and nitric oxide as signalling molecules in plants. Journal of Experimental Botany, 53, 1237–1247.

http://dx.doi.org/10.1093/jxb/53.372.1237

Orabi, S. A., Dawood, M. G., & Salman, S. R. (2015). Comparative study between the physiological role of hydrogen peroxide and salicylic acid in alleviating the harmful effect of low temperature on tomato plants grown under sand-pony culture. Science and Agriculture, 9, 49–59.

http://dx.doi.org/10.1111/ppl.2012.728613

Orozco-Cardenas, M. L., Navaréz-Vásquez, J., & Ryan, C. A. (2001). Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. The Plant Cell Online, 13, 179–191.

http://dx.doi.org/10.1105/tpc.13.1.179

Petrov, V. D., & Breusegem, F. V. (2002). Hydrogen peroxide-a central hub for information flow in plant cells. AoB Plants pls014. doi:10.1093/aobpla/pls014

Potthak, T. S., Collins, C. C., Johnson, D. I., Delmer, D. P., & Levine, M. J. (2005). The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. Plant Physiology, 119, 849–858.

http://dx.doi.org/10.1104/pp.103.3.849

Prochazkova, D., Sairam, R. K., Srivastava, G. C., & Singh, D. V. (2001). Oxidative stress and antioxidative activity as the basis of senescence in maize leaves. Plant Science, 161, 765–771.

http://dx.doi.org/10.1016/S0168-9452(01)00462-9

Qiu, Z. B., Li, Q., Bi, Z. Z., & Yue, M. (2011). Hydrogen peroxide acts as a signal molecule in CO2 laser pretreatment-induced osmotic tolerance in wheat seedlings. Plant Soil and Environment, 57, 403–408.

Quan, L. J., Zhang, B., Shi, W. W., & Li, H. Y. (2008). Hydrogen peroxide in plants: A versatile molecule of the reactive oxygen species network. Journal of Integrative Plant Biology, 50, 2–18.

http://dx.doi.org/10.1111/j.1199-3740.2008.50.issue-1

Ranell, F., Mioloundama, A. S., & Havaux, M. (2012). Non-enzymatic carotenoid oxidation and photooxidative stress signalling in plants. Journal of Experimental Botany. doi:10.1093/jxb/ers223

Rao, M. V., Paliyath, G., Omrod, D. P., Murr, D. P., & Watkins, C. B. (1997). Influence of salicylic acid on H2O2 production, oxidative stress, and H2O2-metabolizing enzymes: Salicylic acid-mediated oxidative damage requires H2O2. Plant Physiology, 115, 137–149.

http://dx.doi.org/10.1111/j.1365-311X.2007.03750.x

Razem, F. (2008). An overview of hydrogen peroxide production and cellular determination in plants. Hebron University Research Journal, 3, 84–96.

Rubio, M. C., James, E. K., Clemente, M. R., Bucciarelli, B., Felpinaya, M., Vance, C. P., & Becana, M. (2004). Localization of superoxide dismutases and hydrogen peroxide in legume root nodules. Molecular Plant-Microbe Interactions, 17, 1294–1305.

http://dx.doi.org/10.1094/MPMI.2004.17.12.1294

Scott, I. M., Dat, J. F., Lopez-Delgado, H., & Foyer, C. H. (1999). Salicylic acid and hydrogen peroxide in abiotic stress signalling in plants. Phytochemistry (austria) special issue. Plant Physiology, 39, 13–17.

http://dx.doi.org/10.1105/tpc.13.1.179

Stemler, A. J. (1997). The case for chloroplast thylakoid carbonic anhydrase. Physiologia Plantarum, 99, 348–353.

http://dx.doi.org/10.1111/j.1399-3054.1997.4ppl.099.ii

Tong, J. C., Hinsinger, P., Drevon, J. J., & Iland, I. A. (2001). Phosphorus deficiency impairs early nodule functioning and enhances proton release in roots of medicago truncatula I. Annals of Botany, 88, 131–138.

http://dx.doi.org/10.1093/obana.2001.1440
Tanou, G., Job, C., Belghazi, M., Molassiotis, A., Diamantidis, G., & Job, D. (2010). Proteomic signatures uncover hydrogen peroxide and nitric oxide cross-talk signaling network in citrus plants. *Journal of Proteome Research, 9*, 5994–6006. http://dx.doi.org/10.1021/pr100782h

Terzi, R., Kadioglu, A., Kalyaciglu, E., & Saglam, A. (2015). Hydrogen peroxide pretreatment induces osmotic stress tolerance by influencing osmolyte and abscisic acid levels in maize leaves. *Journal of Plant Interaction, 9*, 559–565.

Upadhyaya, H., Khan, M. H., & Panda, S. K. (2007). Hydrogen peroxide induces oxidative stress in detached leaves of *Oryza sativa* L. *General and Applied Plant Physiology, 33*, 83–95.

Vandenabeele, S., Van Der Kelen, K. V. D., Dot, J., Gadjiev, I., Boonefoes, T., Morsa, S., ... Van Breusegem, F. V. (2003). A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. *Proceedings of the National Academy of Sciences, 100*, 16113–16118. http://dx.doi.org/10.1073/pnas.2136610100

Veal, E. A., Day, A. M., & Morgan, B. A. (2007). Hydrogen peroxide sensing and signaling. *Molecular Cell, 26*, 1–14. http://dx.doi.org/10.1016/j.molcel.2007.03.016

Yang, J. D., Yuri, J. Y., Zhang, T. H., & Zhao, H. L. (2006). Presoaking with nitric oxide donor SNP alleviates heat shock damages in mung bean leaf discs. *Botanical Studies, 47*, 129–136.

Zelinová, V., Bočová, B., Huttová, J., Mistrík, I., & Tamás, L. (2013). Impact of cadmium and hydrogen peroxide on ascorbate-glutathione recycling enzymes in barley root. *Plant Soil and Environment, 59*, 62–267.

Zhang, X., Zhang, L., Dong, F., Gao, J., Golboith, D. W., & Song, C. P. (2001). Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in vicia faba. *Plant Physiology, 126*, 1438–1448. http://dx.doi.org/10.1104/pp.126.4.1438

Zhang, X. L., Jia, X. F., Yu, B., Gao, Y., & Baj, J. G. (2011). Exogenous hydrogen peroxide influences antioxidant enzyme activity and lipid peroxidation in cucumber leaves at low light. *Scientia Horticulturae, 129*, 656–662. http://dx.doi.org/10.1016/j.scienta.2011.05.009