Scientia Horticulturae 240 (2018) 29–37

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Enhancing seed quality and productivity as well as physio-anatomical responses of pea plants by folic acid and/or hydrogen peroxide application

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ARTICLE INFO

Keywords:
Anatomy
Biofortification
Folic
Hydrogen peroxide
Pea
Yield

ABSTRACT

Two field experiments were carried out at Agricultural Botany Department Experimental Farm, Mansoura University during 2014/2015 and 2015/2016 seasons to assess the role of pre-sowing seed soaking in hydrogen peroxide (0, 2 and 4%) and/or foliar application of folic acid (0, 10 and 20 mg/l) individually and/or in combination on growth, yield, some biochemical constituents and anatomical characters of pea plant (*Pisum sativum*, L. cv Master-B.).

The results indicated that foliar spraying with either folic acid concentrations under control or hydrogen peroxide significantly increased growth parameter, photosynthetic pigment concentration, yield and its components as well as its quality, in special, folic acid concentration of pea seed compared with the control plants. Additionally, application of hydrogen peroxide or folic acid as well as their interactions, improved all anatomical characteristics especially vascular bundle dimension and photosynthetic tissue thickness relative to untreated control plants.

It could be recommended that pre-sowing seed soaking in 2% hydrogen peroxide plus foliar application of 20 mg/l folic acid can be used to induce the plant growth, yield and seed quality of pea plants.

1. Introduction

Currently, over two billion people suffer from “hidden hunger”, a term used to describe malnutrition of micronutrients in special, folic acid (Kennedy et al., 2003). Folic acid (FA) deficiency is an important and understanding problem of micronutrient malnutrition affecting billion of people worldwide associated with neural tube defects (NTDs) as well as cardiovascular disease and cancers (Crider et al., 2011). It has been estimated that NTDs affects > 300.000 pregnancies per annum worldwide being an important cause of prenatal mortality and infantile paralysis (Mallard et al., 2012). In Egypt, no precise statistics on NTDs are available, roughly, the incidence of NDTs was approximately 4.5/1000 live born babies (Temtamy et al., 1998). The life of children with NTDs is very difficult worldwide, a significant high burden of disease and associated costs on families and societies, which become much more magnified in a developing country with limited public social support structures. In Egypt and KSA, it would be much logic and effective to apply the role of prevention better than control, by adopting policies to fight folate deficiency. Enhancing folate content in staple crops “biofortification” is a promising, cost-effective strategy to eradicate folate malnutrition worldwide in rich and poor countries alike (Mayer et al., 2008; Gorelova et al., 2017).

Recently, increasing the interest of botanists in FA has been due to their multiple functions. FA is a central cofactor in the one-carbon transfer, which is involved in many cellular reactions, including nucleotide biosynthesis, amino acid metabolism, photosynthetic CO_2 fixation, and biochemical conversion of nitrogen (Hanson and Gregory, 2011). It can be used as a new, convenient and affordable organic fertilizer to increase the efficiency of the plant and preserve its nutrients (Poudineh et al., 2015). To our knowledge, there are a few reports indicating the role of folic acid application on increasing growth, yield and improving some biochemical constituents in several plants (Rezaee et al., 2012; Vician and Kovacik, 2013).

Pre-sowing seed soaking in an aqueous solution of the chemical producing O_2 like H_2O_2 must be applied for improving seed germination and increases seedling growth for many plants (Liu et al., 2012; Rajashekar and Baek, 2014), depending on its concentration. In general, application of H_2O_2 at low concentration (≤ 2.5 mM) had a stimulatory effect on growth trait, whereas a higher concentration up to 5 mM provokes the onset cell senescence and death (Deng et al., 2012). In this concern, application of H_2O_2 stimulated the growth of some plants (Maswada and Abd El-Rahman, 2014), enhanced the

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https://doi.org/10.1016/j.scienta.2018.05.049
Received 14 October 2017; Received in revised form 29 April 2018; Accepted 25 May 2018

photosynthetic rate and retarded the degradation of chlorophyll (Hasan et al., 2016).

Legumes are the members of the family Fabaceae, which are important crop yielding plants after Poaceae. Pea (*Pisum sativum* L.) is considered as one of the most important sources in human food nutrition throughout the world, due to its high content of protein, carbohydrates, vitamins A, B and ions (Muehlbauer and Mcphee, 1997). The average fresh seed yield of pea in Egypt was 11.640 ton/h during 2015 (FAO Statistics). Modern agriculture aims to produce higher yields, with hitherto little emphasis on the nutritional quality (Sands et al., 2009). So, the present investigation aims to assess the effect of pre-sowing seed soaking in hydrogen peroxide and/or foliar application of folic acid as well as their interactions on growth, some physiological and anatomical characteristics as well as yield and its quality of pea plants.

2. Materials and methods

2.1. Experimental layout and plant management

Field experiments were carried out in Agricultural Botany Department Experimental Farm, Mansoura University (31°02′40.6″N latitude, 31°22′40.3″E longitude and the altitude is 15 m above the sea level), during 2014/2015 and 2015/2016 growing seasons. The experiments were designed to investigate the role of pre-sowing seed soaking in hydrogen peroxide, and/or foliar application of folic acid and their interactions on growth, yield, anatomical characteristics and some biochemical constituents of pea plant (*Pisum sativum*, L. cv. Master-B). The experimental soil was clay-loam in texture with pH (1:2.5) 7.1, 7.2 and 1.15%, 1.29% organic matter content in the first and second seasons.

The experiments were executed in a Completely Randomized Block Design (CRBC) with three replicate. The experiment consists of nine treatments, (1) untreated control plant, (2) seed presoaking in 2% hydrogen peroxide (H_2O_2), (3) seed presoaking in 2% H_2O_2 plus foliar spraying 10 mg/l folic acid (FA), (4) seed presoaking in 2% H_2O_2 plus foliar spraying 20 mg/l FA, (5) seed presoaking in 4% H_2O_2, (6) seed presoaking in 4% H_2O_2 plus foliar spraying 10 mg/l FA, (7) seed presoaking in 4% H_2O_2 plus foliar spraying 20, mg/l FA, (8) foliar application of 10 mg/l FA, and (9) foliar application of 20 mg/l FA; H_2O_2 or folic acid concentrations were chosen based on our earlier preliminary experiment (un-published data). The experimental unit area was 10.5 m^2 which contained three ridges, each 5 m long each with 70 cm wide; one row was left between each to the experimental unit without spraying as a guard row to avoid the overlapping of the spraying solution.

Homogenous pea seeds were surface sterilized with 1% sodium hypochlorite solution for 5 min followed by washing several times with tap water and finally with distilled water. The sterilized seeds were soaked separately for 4 h in hydrogen peroxide percentage (EL-Gomhoria Co. Egypt) as well as distilled water as a control. The treated seeds were dried, then, treated with *Rhizobium leguminosarum* strains, and finally were sown on 2nd November 2014 and 2015 in hill 10 cm apart on one side of each ridge.

The mineral fertilizers were added in a half recommended dose as 75 kg nitrogen/h as ammonium nitrate (33%) and 60 kg K_2O /h in the form of potassium sulphate (48% K_2O). The nitrogen and potassium fertilizers were divided into two equal doses, the first one was added before the first irrigation and the second one was added before the following irrigation. Phosphorous fertilizer was added at 45 kg P_2O_5/h as calcium superphosphate (15.5% P_2O_5), it was added during the experimental field preparation. All other agricultural practices were done at the recommendation of the Ministry of Agriculture, Egypt.

Pea plants were sprayed twice at 30 and 40 days from sowing with folic acid (Sigma Co., USA) at 10 or 20 mg/l and distilled water as a control. Each experimental unit received 2 l spraying solutions. Tween 20 as a wetting agent was added at 0.05%, spraying was done till runoff throughout late afternoon hours using hand atomizer.

2.2. Data recorded

At 55 days from sowing, the plant samples were randomly taken from each experimental unit for assessing plant growth characters, some physiological parameters, and leaflet and stem anatomy.

At the end of the experiments (75 DFS), five plants from each treatment were harvested for determination marketable green pod yield and its components, represented as, pod number per plant, green pod yield per plant, seed number per pod, seed index as a weight of 100 green seeds. Green seeds were taken randomly for determination some quality characteristics (phosphorous and potassium percentage; ascorbic acid and total free amino acids concentration; percentage of protein and carbohydrates, as well as vitamin B_s complex concentrations).

2.3. Plant growth

Five plants from each treatment were removed from the experimental unit for determination plant height, the fresh and dry weight of the shoot, as well as leaf area per plant. Leaf area (cm^2) was measured using leaf area meter, AM 300 (ADC Bioscientific Ltd).

2.4. Physiological Characteristics

Photosynthetic pigment (chlorophylls a, b and their total as well as total carotenoids), were extracted from leaf blade of the 3rd terminal upper compound leaf of the main stem for 24 h at laboratory temperature by methanol after adding a trace from sodium carbonate, then photosynthetic pigments were determined spectrophotometrically (Lichtenthaler and Wellburn, 1985).

Ion percentages were determined in the dried shoot and seed sample after wet digestion with $HClO_3/H_2SO_4$. Total nitrogen was determined by micro-Kjeldahl method. Potassium was determined by a flame photometer (Kalra, 1998), and phosphorous using ammonium molybdate and ascorbic acid (Cooper, 1977). Total protein in the seeds was calculated by multiplying the nitrogen % by 6.25 (Stewart, 1989). Ascorbic acid was extracted from pea seeds and titrated using 2.6- dichlorophenol indophenol as described by Sadasivam and Manickam (1996). Total free amino acid was determined by the method of Chen et al. (2009).

B–Complex vitamins in green seeds were determined in Food Technology Res. Inst., Agric. Res. Centre, Egypt, by the method of Batifoulier et al. (2005), with slight modification using a variable wavelength detector (VWD) instead of fluorescence detector, VWD set at 280 nm. HPLC Agilent 100 series equipped with variable wavelength detector, column compartment set at 35 °C. Quaternary pump, degases and auto samples, ODS column were used to fractionate the components of samples. The mobile phase was potassium phosphate buffer (50 mM. pH 6)/methanol (80/20 v/v), delivered at a flow rate of 1 ml/min. The injection volume was 20 µl and the duration of the analytical run was 10 min. Fluorescence detection was operated at 366 nm excitation and 435 nm emissions.

2.5. Leaflet and stem anatomy

Specimens (5 × 5 mm) from the terminal leaflet of the 3rd upper compound leaf including main mid-vein and the middle part of the 2nd upper internodes of the main stem from the plant tip were taken after 55 days from planting at the second season. The samples were killed and fixed in formalin aceto alcohol for at least 48 h, then washed and dehydrated in series of ethanol and embedded in paraffin wax (52–54 °C melting points). Cross sections were done at 12–15 µm thick using rotary microtome, stained in crystal violet/erythrosine, cleared in