Plant Physiology and Biochemistry 111 (2017) 274–283

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Seed priming improves chilling tolerance in chickpea by modulating germination metabolism, trehalose accumulation and carbon assimilation

Muhammad Farooq [a, b, c, *], Mubshar Hussain [d], Ahmad Nawaz [a], Dong-Jin Lee [e], Salem S. Alghamdi [c], Kadambot H.M. Siddique [b]

[a] Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
[b] The UWA Institute of Agriculture, The University of Western Australia, LB 5005 Perth WA 6001, Australia
[c] College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[d] Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan
[e] Department of Crop Science and Biotechnology, Dankook University, Chungnam, 330-714, South Korea

ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form
28 November 2016
Accepted 7 December 2016
Available online 9 December 2016

Keywords:
α-amylase
Chickpea
Chilling stress
Photosynthesis
Seed priming
Trehalose

ABSTRACT

Chilling stress is one of the major abiotic stresses affecting chickpea productivity worldwide. This study evaluated the potential role of seed priming in improving resistance to chilling stress in chickpea (cv. Punjab, 2008). The priming treatments involved soaking seeds of chickpea cultivar Punjab 2008 in either water for 8 h (on-farm priming), aerated water (hydropriming) for 18 h, or $CaCl_2$ solution (ψ_s −1.25 MPa; osmopriming) for 18 h. Primed and untreated seeds were grown either at 18/15 °C (control) or 13/10 °C (chilling stress). Chilling stress suppressed the growth of chickpea while seed priming mitigated the adverse effects of chilling stress by improving stand establishment, growth, water relations, photosynthesis, α-amylase activity, sugar metabolism, antioxidant enzyme activity, membrane stability, and leaf accumulation of proline, nitrogen, potassium and soluble phenolics. Seed priming also improved the performance of chickpea under optimal (control) conditions. The overall order of improvement in resistance to chilling by using seed priming was osmopriming > hydropriming > on-farm priming. Osmopriming improved seedling dry weight, specific leaf area, leaf CO_2 net assimilation rate, maximal photochemical efficiency of PSII, α-amylase activity, trehalose content and leaf relative water content by 10, 22, 17, 20, 73, 48 and 7%, respectively, relative to the non-primed control under chilling stress. Under optimal temperature conditions, the corresponding values were 30, 32, 16, 10, 83, 75 and 5%, respectively. Sugar metabolism, especially trehalose content, was strongly linked with stand establishment, photosynthesis, antioxidant potential (under chilling stress) and plant biomass. Overall, seed priming improved chickpea performance under both optimal temperature conditions and chilling stress through better germination metabolism and the accumulation of trehalose, which protected from oxidative damage and helped to maintain carbon assimilation and seedling growth.

1. Introduction

The exposure of plants to temperatures below optimal may generate reactive oxygen species (ROS) which interact with several cellular components including DNA, proteins, lipids and pigments (Farooq et al., 2008a, 2009a) which can damage germinating seeds and fully-established plants. Low temperatures may reduce mineral and water uptake, stomatal conductance and photosynthesis (Farooq et al., 2009a; Yadav, 2010) thereby reducing plant growth. Chilling stress directly impacts the photosynthetic apparatus by disturbing thylakoid membranes and chlorophyll pigment

Abbreviations: CUE, coefficient of uniformity of emergence; DNS, dinitrosalicylic acid; EI, emergence index; LSD, least significant difference; ROS, reactive oxygen species; TTC, 2,3,5-triphenyltetrazolium chloride; MDA, malondialdehyde; $^1O_2$, Singlet oxygen; O_2^-, superoxide; H_2O_2, hydrogen peroxide; OH, hydroxyl radical; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; APX, ascorbate peroxidase; ICP, inductively coupled plasma; ψ_w, water potential; ψ_s, osmotic potential; ψ_{s100}, osmotic potential at full turgor.

* Corresponding author. Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.
E-mail address: farooqcp@gmail.com (M. Farooq).

http://dx.doi.org/10.1016/j.plaphy.2016.12.012
0981-9428/

formation (Zhou et al., 2007; Yadav, 2010) due to the enhanced activity of chlorophyllase (Turan and Ekmekci, 2011). Indeed, chilling stress disrupts thylakoid electron transport, the carbon reduction cycle and stomatal control of the carbon dioxide supply, increases sugar accumulation and lipid peroxidation, and disturbs the water balance (Allen and Ort, 2001). In addition, the activity of Calvin cycle enzymes decreases leading to a reduction in utilized photons and increased photoinhibition (Sonoike, 1999). As a result, chlorophyll antenna complexes trap more energy than can be processed biochemically (Ensminger et al., 2006) and thylakoid membranes become over-energized which increases ROS formation—including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) (Ruelland et al., 2009)—which may damage macromolecules. Chilling stress reduces root hydraulic conductivity, which subsequently reduces leaf water status and nutrient availability to plants (Farooq et al., 2009a; Yadav, 2010) and nutrient uptake. However, stomata are unable to close under chilling stress despite the low leaf water status, which further aggravates chilling-induced water stress (Yadav, 2010). Moreover, low temperatures during the reproductive phase can cause flower and pod abortion, and smaller seeds, resulting in substantial grain yield losses (Nayyar et al., 2005).

Plants have evolved many protective defense mechanisms including ROS scavenging systems such as enzymatic antioxidants [superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase and glutathione peroxidase] and non-enzymatic antioxidants [ascorbic acid (vitamin C), glutathione, α-tocopherols (vitamin E), carotenoids and flavonoids] to mitigate the effects of oxidative stress (Verma et al., 2014). Moreover, the accumulation of free proline and soluble phenolics is an important strategy to lessen stress-induced losses in plants (Farooq et al., 2009a, b). Increased accumulation of total and reducing sugars (especially trehalose) provides protection against chilling stress possibly by stabilizing cell membranes, ceasing protein denaturation and acting as a scavenger of free radicals (Benaroudj et al., 2001). Furthermore, the increased buildup of trehalose in plants under stress conditions results in ROS scavenging through hydrogen bonding of its hydroxyl groups to the polar groups of proteins and phosphate groups of membranes (Benaroudj et al., 2001; Farooq et al., 2009b).

Seed priming—a controlled hydration technique that allows pre-germination metabolism without actual germination (Bradford, 1986; Farooq et al., 2006a, b)—is a pragmatic, shotgun approach for improving germination and seedling establishment at sub-optimal temperatures (Farooq et al., 2008b, 2010). Priming also expands the temperature range at which germination can occur (Welbaum and Bradford, 1991; Farooq et al., 2008a, b; 2010). Kaur and associates (Kaur et al., 2003) reported improved germination in chickpea exposed to polyethylene-glycol-induced drought after priming in water (hydropriming) or mannitol (osmopriming) due to significant changes in carbohydrate metabolism.

Calcium (Ca) acts as a second messenger in several signaling cascades in plants (Bush, 1995) and modulates plant responses to several abiotic stresses (Rincon and Hanson, 1986). For instance, chilling stress signaling in tobacco was accompanied by a large transient rise in cytosolic Ca (Knight et al., 1991). Likewise, Minorsky (1989) opined that changes in cytosolic Ca were a necessary step in a temperature-sensing mechanism that enables plants to withstand low-temperature stress. Exogenous application of Ca can improve plant resistance to chilling by increasing cytosolic Ca. For instance, exogenous application of Ca inhibited the loss of chlorophyll under temperature stress by reducing photo-oxidation (Wise and Naylor, 1987) and/or maintaining membrane integrity through increase in the cytosolic Ca (Coria et al., 1998; Farooq et al., 2008a, 2009a). Likewise, Ca application significantly improved Rubisco activity under lower temperature, which was associated with higher rates of photosynthesis (You et al., 2002). The antioxidative defense mechanism, including the production of enzymatic and non-enzymatic antioxidants, helps plants to reduce ROS-induced oxidative damage (Posmyk et al., 2009; Farooq et al., 2009a). Ca application also activates some antioxidant enzymes (Farooq et al., 2008a) and helps to maintain antioxidant activity under temperature stress (Farooq et al., 2008a, 2009a, 2011; Tan et al., 2011).

In rice-based cropping systems and rainfed regions, chickpea (*Cicer arietinum* L.) planting is often delayed due to the late harvest of rice and/or untimely rainfall. In South Aisa a delay in chickpea planting is often tied to low temperatures. Seed priming with Ca salts may improve stand establishment and the productivity of chickpea under a wide temperature range. However, the potential of seed priming with calcium salts to improve the resistance of chickpea to low temperature is rarely reported. It was hypothesized that chickpea seed priming with Ca salts may improve stand establishment and the subsequent growth of chickpea under low temperature. The specific objective of this study was to unravel the mechanism of priming-induced chilling stress tolerance in chickpea.

2. Materials and methods

2.1. Plant material

Seeds of *desi* chickpea cultivar Punjab 2008 obtained from the Pulses Research Institute, Faisalabad, Pakistan were used.

2.2. Seed priming

Seed priming treatments were selected from pre-experiments (data not given). Chickpea seeds were either soaked in tap water for 8 h (on-farm priming), aerated distilled water (hydropriming) for 18 h or $CaCl_2$ solution (ψ_s −1.25 MPa; osmopriming) for 18 h; the seed to solution ratio was 1:5 (w/v). Untreated seeds were used as the non-primed control treatment. Osmoprimed and hydroprimed seeds were then re-dried near to their original weight with forced air under shade and stored at 5 °C until used. On-farm primed seeds were sown immediately after surface drying.

2.3. Experimental details

Primed and non-primed chickpea seeds (six per pot) were sown in acid-washed sand-filled plastic pots (5 kg) and thinned to three plants per pot after the completion of emergence. The pots were placed in one of two climate chambers—18/15 °C (day/night temperature) for the control or 13/10 °C (day/night temperature) for the chilling stress treatment—each with a photosynthetically active photon flux density of 350 mM m^{-2} s^{-1} and a photoperiod of 16/8 h light/dark. The experiment was conducted in a completely randomized design with a factorial arrangement. There were six replicates per treatment and plants were harvested four weeks after planting. A replicate consisted of five pots with three plants per pot, such that there were 90 plants in 30 pots for each of the eight temperature × seed treatments.

2.4. Stand establishment

The number of emerged seedlings was counted daily following the method described by the Association of Official Seed Analysts (1990) until a constant count was achieved. The coefficient of uniformity of emergence (CUE) was calculated using the formula of