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Research article

Salvaging effect of triacontanol on plant growth, thermotolerance, macro-nutrient content, amino acid concentration and modulation of defense hormonal levels under heat stress

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ABSTRACT

In this study, it was hypothesized that application of triacontanol, a ubiquitous saturated primary alcohol, at different times—before (TBHS), mid (TMHS), and after (TAHS) heat stress—will extend heat stress (HS) protection in mungbean. The effect of triacontanol on the levels of defense hormones abscisic acid (ABA) and jasmonic acid (JA) was investigated along with the plant growth promotion, nutrient and amino acid content with and without heat stress. Heat stress caused a prominent reduction in plant growth attributes, nutrient and amino acid content, which were attributed to the decreased level of ABA and JA. However, application of triacontanol, particularly in the TBHS and TMHS treatments, reversed the deleterious effects of HS by showing increased ABA and JA levels that favored the significant increase in plant growth attributes, enhanced nutrient content, and high amount of amino acid. TAHS, a short-term application of triacontanol, also significantly increased ABA and JA levels and thus revealed important information of its association with hormonal modulation. The growth-promoting effect of triacontanol was also confirmed under normal growth conditions. To the best of our knowledge, this study is the first to demonstrate the beneficial effects of triacontanol, with or without heat stress, on mungbean and its interaction with or regulation of the levels of defense hormones.

1. Introduction

The increase in atmospheric CO_2 level due to anthropogenic activities in the last two centuries has raised the surface temperature on the Earth and it is further predicted to increase by 1.1–6.4 °C by the end of this century (Xue et al., 2015). However, even under current conditions, elevated mean temperature has led to highly variable daily or seasonal regional climatic conditions (Dobrá et al., 2015). Variable climatic conditions have often been the major cause of abiotic stresses in crop plants (Rezaei et al., 2015). The increase in the mean high temperature and variable climatic conditions often cause severe heat stress (HS) in crop plants, which greatly hinders growth, development, and productivity (Rezaei et al., 2015; Li et al., 2015; Vignjevic et al., 2015). Heat stress adversely affects important plant physiological processes such as accumulation of reactive oxygen species, the rate and efficiency of photosynthesis, respiration, transpiration, root growth, reproductive organs, assimilate accumulation, and senescence (Barlow et al., 2015; Dou et al., 2015; Rezaei et al., 2015). At the cellular level, HS damages normal cell activities by damaging cell organelles, protein denaturation and aggregation, and then finally causes cell death (Dou et al., 2015; Li et al., 2015).

However, plants have evolved different internal physiological mechanisms at the transcriptomic, proteomic, and metabolomic levels to adjust or avoid prevailing stress conditions (Hasanuzzaman et al., 2013; Dobrá et al., 2015). To further induce HS tolerance, new varieties are constantly developed using biotechnological and molecular approaches to increase

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photosynthetic rates, successful fruiting, and thermostability (Bita and Gerats, 2013; Hasanuzzaman et al., 2013). Exploitation of microorganisms like symbiotic fungal endophytes is another option to confer HS tolerance (Waqas et al., 2015a). Another strategy is the exogenous application of heat stress protectant compounds, which have been developed and recommended on the basis of their role in plant growth and development. There are numerous protectant compounds which have a diversity of functions e.g. osmolytes, plant growth hormones, small and low molecular mass signaling molecules, and trace elements (Hasanuzzaman et al., 2013). Triacontanol has been identified as one of the potent biostimulants (Chen et al., 2003; Ertani et al., 2013), and is a natural long chain C-30 primary alcohol present in epicuticular waxes of plant upper surfaces (Chen et al., 2003, 2005). Different plant growth regulatory and physiological roles have been identified for triacontanol ranging from enhancing shoot growth, induction of early flowering, and modification of photosynthetic rates (Chen et al., 2005). Triacontanol has been reported to stimulate plant growth and increase stress resistance against salinity, drought, and acidic mist stress (Laughlin et al., 1983; Naeem et al., 2012). Under these abiotic stresses, triacontanol application has been reported to prevent oxidative stress, breakdown of enzymatic and non-enzymatic lipid peroxidation in chloroplast thylakoid membranes, inhibit stress related genes while up-regulates genes involved in modulation of various physiological and biochemical functions (Naeem et al., 2012). Apart from these important functions, triacontanol has been used as protectant against heavy metals toxicity and enhances the bio-removal process of pollutants such as boron from wastewater in microalgae (Taştan et al., 2012). In addition, triacontanol improved the nutrient, amino acid, chlorophyll, reducing sugars, starch and relative water content, photosynthesis and respiration under various kinds of abiotic stresses (Naeem et al., 2012; Ertani et al., 2013; Perveen et al., 2014). Under most of the abiotic stresses, plants exhibit this common characteristic to alleviate or escape stress and therefore accordingly regulate different mechanisms like photosynthesis, hormonal regulation and nutrient uptake; hence triacontanol was thought to prove its effectiveness in HS as it showed in other abiotic stresses.

Therefore, in the current study, it was hypothesized that application of triacontanol in mungbean under prolonged HS will modulate the physiological and biochemical functions to rescue growth. Mungbean (*Vigna radiata* (L.) R. Wilczek) is a short duration annual legume crop mostly grown in South and Southeast Asia under rain-fed conditions (Waqas et al., 2014a; Nair et al., 2015; Yao et al., 2015). Usually the seed of the mungbean is consumed for variety of human food purposes, either cooked as a curry known as 'dhal' or roasted with spices to serve as a snack (Waqas et al., 2014a). The green foliage is also utilized as animal feed or the standing crop is buried in soil for green manure (Nair et al., 2015). Different bioactive properties of mungbean have been identified such as antitumor, antioxidant, and antidiabetic properties (Yao et al., 2015). Besides these beneficial bioactive properties, mungbean is a rich source of dietary protein (24–28%), carbohydrate (59–69%), energy (3400 KJ energy/Kg grain), as it is easily digestible and has low phytic acid and high iron content (Nair et al., 2015). Because most mungbean cultivation is confined to rain-fed areas, production is likely hindered by severe heat stresses (Singh and Singh, 2011; Waqas et al., 2014a; Nair et al., 2015). Hence, this study was conducted with the following objectives: 1) to determine the effect of prolonged heat stress on mungbean, 2) to determine the growth promoting and rescuing effect of triacontanol application on mungbean with and without heat stress, 3) to study the phytohormonal levels of ABA and JA with and without heat stress and their response to triacontanol application, 4) to study amino acid and macro nutrient content with and without heat stress and triacontanol interaction.

2. Materials and methods

2.1. Plant materials and growth under controlled conditions

Mungbean seeds (*Vigna radiata* (L.), Wilczek) were commercially obtained from SUNGWOO SEED (Seoul, South Korea). The seeds were healthy, with 6% moisture content and 100% germination rate. Concurrently to pot preparation, seeds were germinated (28 °C and relative humidity of 60%) for six days in germination trays filled with microbial free bio-soil (Dongbu Farm Hannong, South Korea) to obtain seedlings of equal size. Before germination, the seeds were surface sterilized in autoclaved pots with 2.5% sodium hypochlorite for 30 min, and were then rinsed with autoclaved double-distilled water. After germination, seedlings of equal size were randomly selected and transplanted to pots. Plants were left to grow for 17 days under controlled growth chamber (KGC-175 VH, KOENCON) conditions (day/night cycle: 14 h at 30 °C/10 h at 23 °C; relative humidity 60–70%; light intensity 1000 μE m^{-2} s^{-1} from sodium lamps). The mungbean were irrigated daily as per requirement with 30 ml freshly autoclaved DDW to minimize nutrient leaching from pots. After 17 days of growth, mungbean plants were randomly divided into two experimental treatment units. The selected plants were sprayed with triacontanol or a control with a mock solution and were left for six days before heat stress. After six days of growth with and without triacontanol, plants were further divided into two groups: A) no stress (NS) with (1) mock treatment (CNS), and (2) treatment with 11 μM triacontanol (TNS); B) heat stress (HS) with (1) mock treatment (CHS), (2) treatment with 11 μM triacontanol before HS (TBHS), (3) treatment with 11 μM triacontanol at the midpoint of the HS treatment (TMHS), and (4) treatment with 11 μM triacontanol after HS (TAHS). The triacontanol concentration (11 μM) was selected on the basis of previous literature as reported by Ertani et al. (2013). Triacontanol or mock solution (20.8 ml) was thoroughly applied by a hand spray machine on each mungbean plant on both sides of leaves and was considered sufficient when the solution tickled down as droplets from wet leaves. In order to reach the desired triacontanol (96%, w/v; Sigma–Aldrich, USA) concentration (11 μM/5.03 mgL^{-1}), it was initially dissolved in chloroform with a few drops of Tween 80 and brought to the final volume by adding double distilled water. For the mock (control) treatment, an equal amount of chloroform, Tween 80, and water were used as in the treatment solution without the addition of triacontanol.

2.2. Heat stress application

Mungbean plants in the HS group with and without triacontanol treatments were subjected to heat stress for one week. In the HS treatment, the same growth chamber conditions were maintained, except for temperature, which was adjusted to 40 °C/38 °C (day/night cycle). After HS, the mungbean plants in CHS, TBHS, and TMHS treatments along with those without heat stress (CNS and TNS) were harvested and immediately stored in liquid nitrogen and then freeze-dried for 1 week (ISE Bondiro freeze dryer, Ilsin Bio Base, Yangju, South Korea). However, plants designated for TAHS were left for 2 h after spraying with triacontanol and then collected and processed in the same manner as the other treatments.

2.3. Plant growth data

Before harvesting, the shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), root fresh weight (RFW), root dry weight (RDW), number of branches $plant^{-1}$ (NBP),