



Silicon fertilization modulates 2-acetyl-1-pyrroline content, yield formation and grain quality of aromatic rice



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ARTICLE INFO

Article history:

Received 24 August 2016

Accepted 13 March 2017

Available online 22 March 2017

Keywords:

Fragrant rice

Growth

Proline

2-Acetyl-1-pyrroline

Silicon

Yield

ABSTRACT

Present study aimed to assess silicon (Si) mediated yield, grain quality and regulations in 2-acetyl-1-pyrroline accumulation (2-AP) in aromatic rice. Four different levels of Si at 15, 30, 45 and 60 mg kg⁻¹ were applied to two aromatic rice cultivars i.e., Nongxiang 18 and Meixiangzhan 2, while pots without Si were served as control (CK). Results revealed that Si fertilization improved 2-AP, Si and proline contents in leaves and grains as well as activities of proline dehydrogenase (PRODH) and net photosynthetic rates (Pn) (in leaves) while interfered with total N contents in leaves and grains. Moreover, leaves N and proline contents, and net photosynthetic rates (Pn) were decreased with plant age i.e., tillering > flowering > maturity while PRODH activities and Si contents were highest at flowering and maturity stages, respectively and minimum at tillering stage. Furthermore, growth, yield and quality components were also improved by Si application but results were not consistent regarding grain quality for both rice cultivars. Further, Si contents in leaves have significant positive relations ($r = 0.3974$, $P < 0.05$) with grain 2-AP contents at flowering stage. Hence, Si proved better for both rice cultivars whereas 2-AP contents were higher for Meixiangzhan 2 than Nongxiang 18.

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1. Introduction

Fragrant rice constitutes a special subgroup of rice that is largely eminent in whole world for its unique 'popcorn-like' or 'nutty' flavor. Pakistani and Indian 'Basmati' and Thai 'Jasmine' are premium fragrant rice cultivars which are world famous due to their typical long-grain and fragrance in both raw and cooked state. Currently, the fragrant cultivars are fetching very high prices in the world rice markets while consumer demand for fragrant rice is also increasing over the world (Mo et al., 2015). Originally these fragrant rice varieties were extensively cultivated and produced in only three main countries, i.e., Pakistan, India, and Thailand, but currently they are scattered in many parts of the world (Nadaf et al., 2014).

From decades, the extensive studies relating the isolation and characterization of aroma compound from these fragrant rice

cultivars have been put through. More than 300 volatiles have been identified from different fragrant and non-fragrant rice (Maraval et al., 2008), of which 2-acetyl-1-pyrroline (2-AP) has been identified as a key flavoring compound in fragrant rice that distinguishes it from other non-fragrant varieties (Maraval et al., 2008). The 2-AP is generated inside the leaf, stem and rice kernel during plant development (Yoshihashi, 2002), however, non-fragrant rice also accumulate 2-AP in above ground plant parts, but its concentrations are significantly lower than those found in fragrant rice. Hence, the presence of the 2-AP content is considered a major feature of rice aroma quality.

The factors affecting 2-AP biosynthesis are equally complex from gene expression to environmental factors and crop management practices (Gay et al., 2010; Li et al., 2016). Although, the genetic factors play a pivotal role in determining the quality of fragrance rice (Bradbury et al., 2008), but still it is very much dependent on prevailing environmental conditions during growth cycle and also on pre and post-harvest management techniques (Gay et al., 2010). Some other factors such as temperature during grain filling and ripening, plant nutrition and fertilizer application,

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cultivation practices, soil type and finally the storage and processing conditions may also affect the aroma in rice grains. Furthermore, abiotic stresses i.e., drought and/or salinity, may also have a positive impact on 2-AP content in the rice grains at harvest (Gay et al., 2010). Previous studies showed that betaine aldehyde dehydrogenase (BADH) leads to the formation of γ -aminobutyric acid (GABA) through γ -aminobutyraldehyde, while the inactivation of BADH in fragrant rice leads to the formation of Δ^1 -pyrroline through γ -aminobutyraldehyde (Bradbury et al., 2008). Recently, Mo et al. (2015) found significant correlations of grain 2-AP with GABA contents in fragrant rice under shading during grain-filling stage. Furthermore, variation in 2-AP contents due to plant growth regulators, nutrient supplementation, planting density, sowing/harvesting time and storage conditions has also been well reported (Goufo et al., 2011; Yang et al., 2014; Mo et al., 2016; Li et al., 2016). In addition to 2-AP, plant nutrition and other plant factors is also involved in regulating the grain quality attributes. For example, distribution of amino acids, proteins and chain length of amylopectin are largely affected by N fertilizer in rice (Yang et al., 2016; Ning et al., 2010) whereas rice panicle morphology could also regulate the grain amylose contents in rice (Cheng et al., 2007).

Silicon (Si) is the most abundant element in the earth crust after oxygen. Although it is considered as non-essential mineral for most of the plants but it has direct or indirect effects on plant growth and development. It plays significant roles in plant growth, nutritional balance, physio-biological functions and stress tolerance mechanisms against both biotic and abiotic stresses. Generally, plants utilize the accumulated silicon for protection against different biotic and abiotic stresses, for example, under field conditions the silicon fertilization also helps in improving resistance to lodging, increasing the erectness of leaves; thus allowing better light transmittance through plant canopies and indirectly improving whole-plant photosynthesis and ultimately rice yield (Tamai and Ma, 2008). The literature also confirms that the silicification not only have substantial effects on grain yield but also brings significant improvements in quality characters of rice (Zhang et al., 2007).

Above literature signifies that Si fertilization to crop plants can affect growth, yield and grain quality, by directly affecting their photosynthetic and physiological characteristics. To date, no study has yet been reported regarding effects of Si application on 2-AP biosynthesis while a little is known about roles of Si in improving grain yield and quality attributes of aromatic rice. This study investigated the effects of Si application on 2-AP biosynthesis, yield and quality characters in fragrant rice. Furthermore, except growth and yield, quantification of proline, a precursor of 2-AP, with assessment of grain Si and N contents, activities of proline dehydrogenase (PRODH) in different plant parts at different growth stages were also studied to provide better insights into the effects of Si on aroma formation in fragrant rice.

2. Materials and methods

2.1. Plant materials and experimental details

The seeds of two aromatic rice cultivars i.e., Nongxiang 18 and Meixiangzhan 2 were collected from College of Agriculture, South China Agricultural University, Guangzhou, China. These two rice cultivars are popular aromatic rice cultivars and well-adopted on double rice cropping system in South China. Moreover, Meixiangzhan 2 developed from (Lemont (USA) \times Fengaozhan), a thermal type conventional rice varieties develop by Rice Research Institute, Guangdong Academy of Agricultural Sciences in 2006 whereas Nongxiang 18 (nongxiang 16 \times Sanhezhan) is a high quality aromatic rice cultivar developed by Rice Research Institute of Hunan province in 2010. Before sowing, seeds were soaked in water for 24 h and then

allowed to germinate in dark chamber at 30 °C for next 24 h. Nursery for both cultivars were raised at Research Farm of College of Agriculture, South China Agricultural University, Guangzhou (23°09'N, 113°22'E and 11 m above the sea level). The region has a humid subtropical monsoon type of climate characterized by warm winters and hot summers with yearly average temperature range lies between 21 and 29 °C (Li et al., 2016).

After 30 days of sowing, seedlings of both rice cultivars were manually transplanted to the soil-filled plastic pots (5 hills per pot and 3 seedlings per hill). Each pot was filled with 12.5 kg of air dried soil after passing it through a 10-mesh sieve. Flooded conditions (3–4 cm water layer above soil surface) were maintained during the whole crop growth period. The experimental soil (0–20 cm) was collected from the fields that are under paddy cultivation from many years. After air drying and passing through 2-mm sieve, soil sample was analyzed to determine physico-chemical properties and “available silica”. The sample was extracted by using the citric acid solution, while the soil leaching liquor was used for determination of “available silica” by the colorimetric molybdenum blue method at 600 nm. The experimental soil was sandy loam containing 23.34 g kg⁻¹ organic matter, 6.14 pH, total nitrogen 1.139 g kg⁻¹, total phosphorus 1.136 g kg⁻¹, total potassium 24.41 g kg⁻¹, alkali-hydrolyzable nitrogen 114.27 mg kg⁻¹, available potassium 61.34 mg kg⁻¹, available phosphate 127.03 mg kg⁻¹ and available silica (water soluble) 51.30 g kg⁻¹.

2.2. Silicon treatments

A special silicon fertilizer containing $\geq 20\%$ of soluble SiO₂ (free from any other nutrient element) was obtained from Farmers' professional cooperative of Ruinong Cotton, Nangong, China, and used in this study. Experiment contained four Si treatments i.e., T1: SiO₂ at 15 mg kg⁻¹ (Silicon fertilizer 75 mg kg⁻¹); T2: applied SiO₂ 30 mg kg⁻¹ (Silicon fertilizer 150 mg kg⁻¹); T3: applied SiO₂ 45 mg kg⁻¹ (Silicon fertilizer 225 mg kg⁻¹); T4: applied SiO₂ 60 mg kg⁻¹ (Silicon fertilizer 300 mg kg⁻¹). Pots without Si-specialized fertilizer were served as control (CK). These levels of Si were based on previous reports of Dai et al. (2009) who conducted an experiment by using silicon fertilizer at 0–120 kg ha⁻¹ and found that applied silicon fertilizer at 90 kg ha⁻¹ is optimum for better growth of rice with maximum produce. Each pot was applied with 181 mg kg⁻¹ urea (46% N), 333 mg kg⁻¹ calcium superphosphate (12% P₂O₅) and 117 mg kg⁻¹ potassium chloride (60% K₂O) at two times with 60% at basal and 40% at tillering stage.

2.3. Sampling and measurement

2.3.1. Determination of proline contents in leaves and grains

At tillering stage fifteen top fully expanded leaves, at flowering stage and maturity, fifteen flag leaves of the main stem, and at maturity fifteen panicles were sampled carefully and stored at –80 °C after treated with the liquid nitrogen for determination of proline content in leaves and grains and proline dehydrogenase (PRODH) activity in leaves.

The proline content was determined according to the method described by Bates et al. (1973). The absorbance of the red chromophore in the toluene fraction was measured by using the UV–VIS Spectrophotometer UV-2550 (Shimadzu, Japan) at 520 nm and the amount of proline was determined by comparison with a standard curve and expressed as $\mu\text{g g}^{-1}$.

2.3.2. Determination of proline dehydrogenase (PRODH, EC 1.5.99.8) activity in leaves

Proline dehydrogenase (PRODH) activity in the leaves was measured as described by Ncube et al. (2013). The absorbance of

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