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Comparative Study on Growth Performance of Transgenic (Over-Expressed *OsNHX1*) and Wild-Type Nipponbare under Different Salinity Regimes

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Abstract: Transgenic Nipponbare which over-expressed a Na^+/H^+ antiporter gene *OsNHX1* was used to compare its growth performance, water status and photosynthetic efficiency with its wild type under varying salinity regimes. Chlorophyll content, quantum yield and photosynthetic rate were measured to assess the impact of salinity stress on photosynthetic efficiency for transgenic and wild-type Nipponbare. Effects of salinity on water status and gas exchange to both lines were studied by measuring water use efficiency, instantaneous transpiration rate and stomatal conductance. Dry shoot weight and leaf area were determined after three months of growth to assess the impacts of salinity on the growth of those two lines. Our study showed that both lines were affected by salinity stress, however, the transgenic line showed higher photosynthetic efficiency, better utilization of water, and better growth due to low transpiration rate and stomatal conductance. Reduction of photosynthetic efficiency exhibited by the wild-type Nipponbare was correlated to its poor growth under salinity stress.

Key words: growth performance; salinity stress; Na^+/H^+ antiporter gene *OsNHX1*; transgenic rice; photosynthetic efficiency; water status

Salinity is one of the major environmental stress that can affect photosynthetic performance, growth and yield of rice. Changes in physiology in any plants caused by salt stress may affect overall growth of the plant which eventually cause low yield and thus reduce rice production as a whole (Aslam et al, 1993; Chowdhury et al, 1995; Sohn et al, 2005; Khan and Panda, 2008; Cha-um et al, 2009).

Increasing sea levels caused by global warming and frequent flooding which lead to high tide would cause an increase in soil salinity as happened in Mekong Delta (Ozaki et al, 2014). In Tanjong Maya (Tutong District, 4°45′50″ N, 114°39′6″ E), due to seepage of saline water, the whole paddy cultivation area is not suitable for growing rice and the land has been now converted to Rumbia (*Metroxylon sago*) tree cultivation (Yunos, 2010).

One possible way to make those lands viable for rice cultivation is introduction of rice varieties with tolerance to salinity. Salt-tolerant rice can be produced using biotechnology by expressing a Na^+/H^+ antiporter gene *OsNHX1* into salt-sensitive rice varieties (Fukuda et al, 2004). Vacuolar Na^+/H^+ antiporters are thought to be responsible for salt tolerance in plants (Blumwald et al, 2000; Fukuda et al, 2004). Blumwald et al (2000) confirmed that the transfer of Na^+ from cytosol or cytoplasm into vacuoles is driven by Na^+/H^+ antiporter using electrochemical gradient of protons generated by vacuolar H^+-translocating enzymes, H^+-ATPase and H^+-PPiase. Salt-tolerant species tend to accumulate large amount of Na^+ in the vacuoles (Bjorkman and Demmig, 1987; Maxwell and Johnson, 2000). This eventually allows more water to be driven into cell, thus increasing salinity tolerance in rice. By

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expressing this antiporter gene into salt-sensitive rice variety, the transgenic rice will show better tolerance to salinity resembling the salt-tolerant rice variety.

According to Fukuda et al (2004) and Islam and Seraj (2009), the expression of *OsNHX1* is controlled by salt stress and exposing rice plants with high salt level will increase transcript level of *OsNHX1* in rice roots and shoots. With frequent transcription of this gene, the expression of Na^+/H^+ antiporter gene will increase and eventually improve rice salt tolerance.

Oryza sativa L. cv. Nipponbare is a temperate japonica rice variety which is known to be sensitive to salt stress. Growth of this variety is inhibited when exposed to high salinity stress. However, the salinity tolerance of the transgenic Nipponbare is improved after introduction of *OsNHX1* into its genome (Fukuda et al, 2004). The transgenic Bangladeshi rice variety Binnatoa with *OsNHX1* produced by Islam et al (2009) also showed significantly higher yield compared to its wild type.

Chen et al (2007) and Faiyue (2011) reported that the transgenic rice line of *O. sativa* L. cv. IRAT109 showed improvement in salinity tolerance after introducing *OsNHX1*. Damage or death appearance caused by salinity stress was delayed in this line. The control plants of the wild type gradually wilted in 4 d when exposed to 200 mmol/L NaCl and eventually died after one week. In comparison, the transgenic line wilted 3–4 d after the control wilted and was able to survive for another two weeks. The osmotic potentials of transgenic plants were lower than those of the control, inferring that the transgenic lines had absorbed more Na^+ in their vacuoles, which would allow the transgenic lines to absorb more water from its surrounding.

Overall, transgenic rice which over-expressed Na^+/H^+ antiporter gene shows improvements in salinity tolerance than its wild type. This may help to increase yield of salt-sensitive rice varieties even when experiencing salinity stress. In this investigation, we compared growth performance, photosynthetic efficiency and water status between transgenic (over-expressed antiporter gene *OsNHX1*) and wild-type Nipponbare grown under different salinity regimes to evaluate the best conditions under which the variety could be cultivated in Brunei Darussalam.

MATERIALS AND METHODS

Salinity treatments of plants in soil

Transgenic (over-expressed *OsNHX1*) and wild-type Nipponbare plants were grown in pots (16 cm in height and 20 cm in diameter) for three months at greenhouse (Universiti Brunei Darussalam) with an average of 286 μmol/(m^2·s) light intensity, 66% relative humidity and 32.6 °C environmental temperature. Each pot comprised of five plants (transgenic or wild-type Nipponbare) was subjected to salinity treatments. Three pots were prepared for each salinity regimes of 0 (control), 50, 150 and 300 mmol/L. Randomized block design described by Cha-um et al (2009) was employed (uniform microhabitat conditions were provided for all pots). Twice a week, 500 mL the saline solution (0, 50, 150 or 300 mmol/L) and 50 mL half strength Hoagland's solution (Taiz and Zeiger, 2002) were supplied to respective pot. The seedlings were planted till the maturity stage starting from October to December 2013. Gathering of physiological measurements (photosynthetic efficiency, leaf gas exchange and water status) and growth data were commenced 3 d after the first salinity treatment and continued for two months at a 4 d interval (Zhao et al, 2006a).

Chlorophyll fluorescence and chlorophyll content measurements

Chlorophyll content was measured twice a week for two months using a chlorophyll content meter-200 (Opti-Sciences, Tyngsboro, Massachusetts, USA). Measurement was taken from the same youngest fully extended leaf following the method described by Wankhade et al (2013). This parameter was measured six times from the leaf tip to base, and the average was used for assessing the chlorophyll content in the leaf sample (Jamil et al, 2014).

Quantum yield (Fv/Fm) was measured using Fluorpen FP100 (Photon Systems Instruments, Brno, Czech Republic). Measurements were taken from the same leaf as for chlorophyll content measurement at three different locations (at the leaf base, middle and near the leaf tip). Prior to the measurement, the leaf was dark-adapted with aluminium foil for 30 min (Wankhade et al, 2013).

Leaf gas exchange and water status

Stomatal conductance, transpiration rate and photosynthetic rate were measured using a LI-6400XT Portable Photosynthesis System with light emitting diode chamber (LI-COR Inc, Lincoln, Nebraska, USA) in three plants per pot for each line and salinity regime. These measurements were taken in the morning till midday (8:00 am to 2:00 pm) from the youngest fully