A Progressive on Salt-tolerant Rice Improvement Using Plant Biotechnology in Thailand

Suriyan Cha-um[1], Sukanya Srithandon, Praderm Wanitchananan, Chalermpol Kirdmanee

[1]National Center for Genetic Engineering and Biotechnology, www.biotec.or.th Email suriyanc@biotec.or.th

Abstract

Salinity of land causes one of the main abiotic stresses that directly affect crop growth and productivity, especially rice crops. Rice has been reported as being salt sensitive, while it serves as a major carbohydrate source for the world's population, mainly in Asian countries. In Thailand, the most popular rice variety is Thai jasmine rice (KDML105), which has an aromatic flavor, a high cooking quality, softness and long grains. The major cultivation area for KDML105 is located in the Northeastern region of Thailand. This is a large area which is affected by salinity problems caused by underground rock salt, leading to low crop yields. Salt tolerant rice has been progressively developed by several research groups. The objective of this investigation was to improve the salt tolerant rice to be cultivated on the land affected by salinity. Homjan (HJ) is a salt-tolerant Thai rice variety grown in the Southern part of Thailand near the sea. It is an effective variety to use as parental lines in salt-tolerant rice breeding programs. Conventional breeding between HJ and KDML105 in reciprocal crosses was practically applied to produce a mass population. The salt-tolerant abilities of rice populations were screened using multivariate parameters, biochemical and physiological characters, under an *in-vitro* environmental control system. Screening was rapid, using accurate and reproducible procedures. In addition, pure line production of salt-tolerant lines was developed using anther culture technology.

Media summary

The genetic resource of salt tolerant rice was found and made a conventional breeding with jasmine rice. The salt tolerant lines were classified as well as pure line production was developed through anther culture. The field adaptation to salinity in pure lines will be tested in several locations of Northeast Thailand before the release of the salt-tolerant rice varieties.

Key Words

Glycinebetaine, Na^+ accumulation, net photosynthetic rate, *Oryza sativa* spp. *indica*, osmoregulation, water relation

Introduction

Rice is a major crop, which is the staple food for more than 3 billion people and provides 50-80% daily calorie intake (Khush 2005). Salinity soil is one of the most important barriers to reduce on rice growth and developments (Zeng et al. 2003). Na^+ ions are well known the toxic damages to plant cells in both ionic and osmotic effects, causing on growth retardants and low productivity prior to death (Mansour and Salama 2004; Chinnusamy et al. 2005). In case of rice, there is reported as salt-sensitive and presented the negative effects of salinity on seedling and reproductive stages (Khan and Abdullah 2003; Zeng et al. 2003). There are many research groups to find-out the salt-tolerant rice from the genetic resources or gene bank using multivariate screening system in both growth and yield performances (Zeng et al. 2004). In addition, anther culture is one of short-cut biotechnologies for pure line production. This technique composes of two steps, including the induction of embryogenic calli from microspores and the green plantlet regeneration from the embryogenic calli (Lentini et al., 1995; Raina and Zapata, 1997). Subsequently, plantlet regeneration from embryogenic callus derived-anthers has rather been reported as large barriers in term of low green plants, high albino plantlets and poor callus development (Jahne and Lorz, 1995; Lentini et al., 1995). In previous studies, the polyamine adding media plays a role as the major factor to enhance on rice anther culture technology (Rajam and Yadev, 1997). The aim of this investigation was to improve the salt tolerant rice to be cultivated on the salinity land.

Methods

Development of salt-tolerant indices

Seeds of jasmine rice (KDML105) salt-sensitive and Homjan (HJ) salt-tolerant varieties (GS No. 4371) were used as the initial plant materials. *In-vitro* seedlings (21 day-olds) grown under photoautotrophic conditions were treated with various NaCl salt concentrations (Fig. 1). Sodium ion (Na^+) and glycine betaine in salt-stressed seedlings were assayed as biochemical changes according to Dionisio-Sese and Tabita (1998) and Gorham (1996), respectively. Photosynthetic pigments and net photosynthetic rate were measured as physiological parameters, following Shabala et al. (1998) and Cha-um et al. (2006), correspondingly.

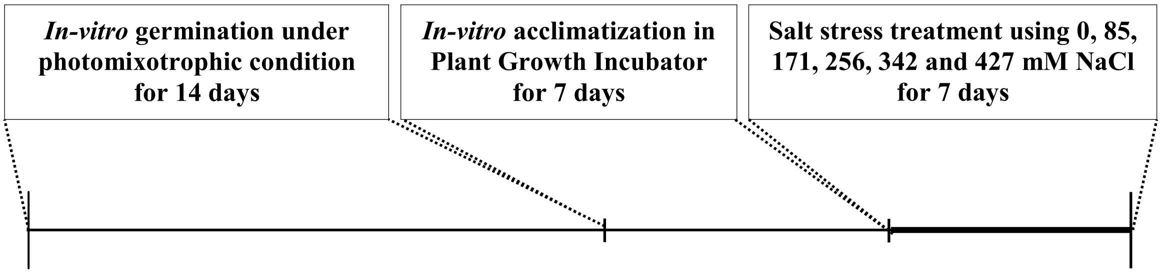

Fig. 1 Scheme of the experiment on *in-vitro* photomixotrophic germination for 14 days, photoautotrophic acclimatization for 7 days and subsequently exposed to 0, 85, 171, 256, 342, and 427 mM NaCl for 7 days.

Conventional breeding and salt-tolerant classification

KDML105 and HJ rice were conventionally breed using reciprocal crosses. Rice lines were collected, *in-vitro* germinated and micropropagated. Biochemical and physiological characters in salt stressed plantlets were collected. Both biochemical and physiological reduction percentages were calculated and then input to SPSS software for cluster analysis using Hierarchical Cluster to be classified the salt tolerant or salt sensitive varieties (Cha-um et al. 2007).

Rice anther culture and pure line production

Salt tolerance of rice lines was cultivated in the rice field prior to flowering initiation. Panicles at the booting stage were collected and disinfected using Clorox®. Rice anthers were excised and inoculated on semisolid modified-N_6 medium supplemented with 2.30 µM kinetin, 4.50 µM 2,4-Dichlorophenoxy acetic acid (2,4-D) and 13.50 µM Naphthalene acetic acid (NAA) for calli induction. Embryogenic callus derived from anther culture was transferred to culture on fresh MS medium supplemented with 9.3 µM kinetin, 5.4 µM NAA, 87.6 mM sucrose and 0.5 mM putrescine (Put) for plantlet regeneration. Pure lines of salt tolerant rice should be tested in several locations of Northeast Thailand.

Results

Sodium ion (Na^+) in KDML105 seedlings grown under salt stress was progressively accumulated and higher than that in HJ salt tolerance (Fig. 1A). On the other hand, glycine betaine (Glybet) in salt stressed HJ rice was reach higher than that in salt stressed KDML105 rice (Fig. 1B). The accumulation of Glybet in the HJ rice may play a role as osmoregulation defense mechanism when expose to salt stress. Total chlorophyll contents and net photosynthetic rate in salt stressed HJ rice were gradually decreased, while those in salt stressed KDML105 rice were sharply reduced (Fig. 2). The HJ rice was an effective salt-tolerant genetic resource to select as a parental line to conventionally breed with KDML105 for salt tolerant improvement. The progenies of reciprocal crosses were classified the salt tolerance group, HJ, 21 and Pok, and salt sensitive group, 26, 409, KDML105, 306, 31, 20, IR29, 598, 18 and 2 (Fig. 3). The salt tolerant line, 21, was selected to produce a pure line using anther culture technique. Green plantlets derived from anther culture were successfully developed (Fig. 4). Furthermore, the pure line production of salt tolerant rice will be promptly planted in the salinity field trial.

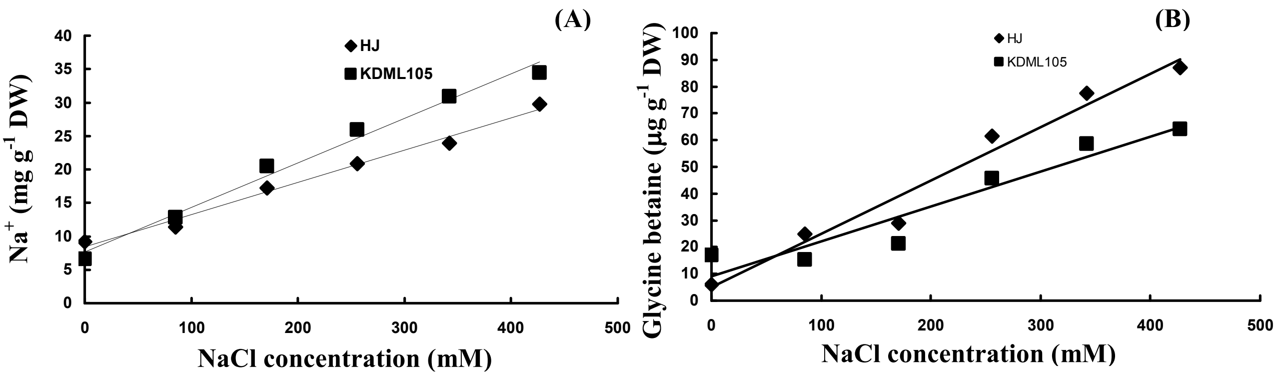

Fig. 2 Sodium ion (A) and glycine betaine accumulations in Homjan (HJ) and jasmine rice (KDML105) grown in photoautotrophic conditions supplemented with various NaCl concentrations for 7 days.

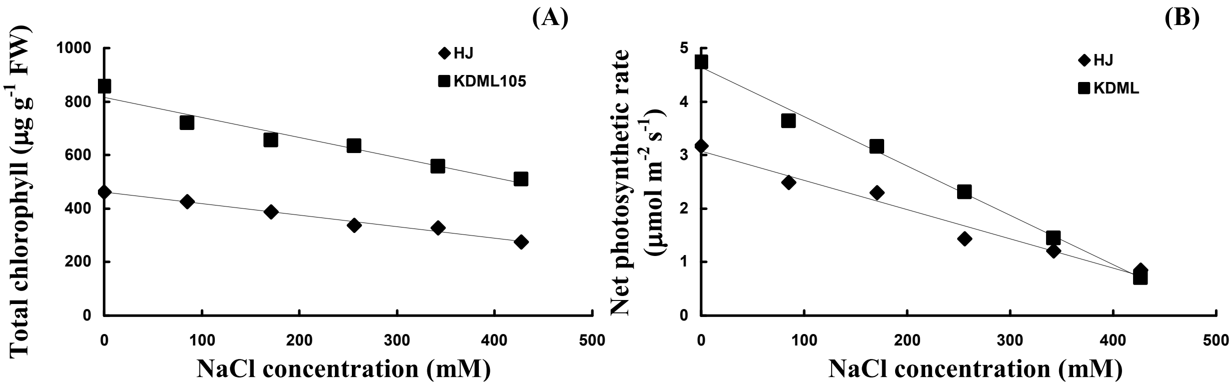

Fig. 3 Total chlorophyll content (A) and net photosynthetic rate (B) in Homjan (HJ) and jasmine rice (KDML105) grown in photoautotrophic conditions supplemented with various NaCl concentrations for 7 days.

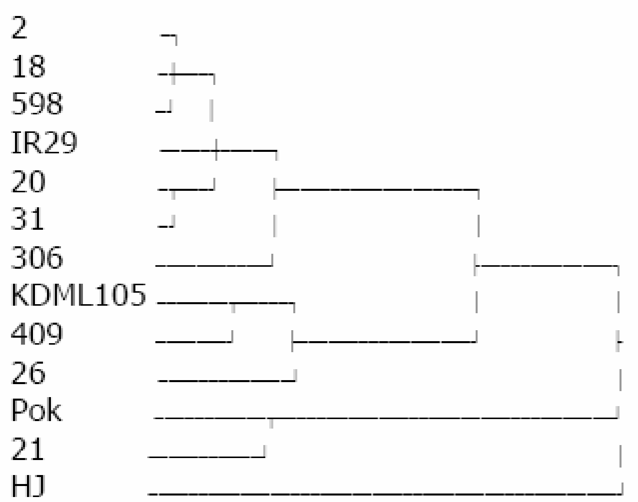

Fig. 4 Cluster analysis of reciprocal cross population in term of salt tolerant class, HJ, 21 and Pok, and salt sensitive class, 26, 409, KDML105, 306, 31, 20, IR29, 598, 18 and 2, in rice seedlings using multivariate parameters of biochemical and physiological parameters by Hierarchical cluster analysis of SPSS software.

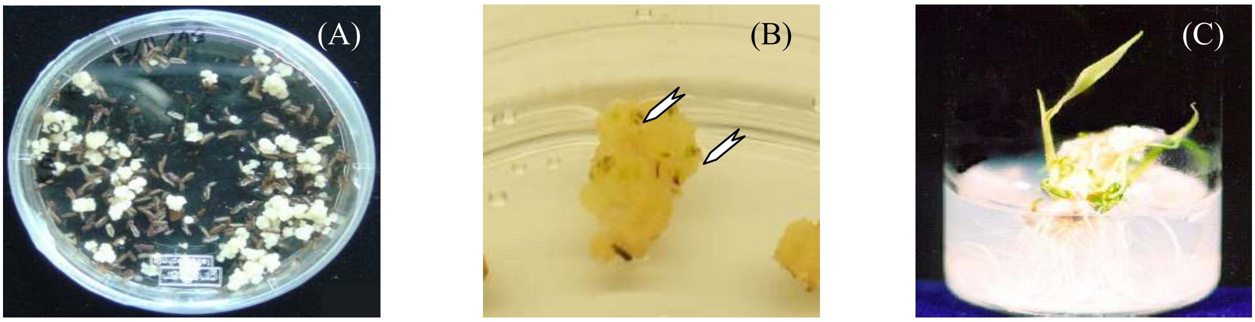

Fig. 5 Calli induction (A), green spot initiation from embryogenic callus (B) on MS medium containing 9.3 µM kinetin, 5.4 µM NAA, 87.6 mM sucrose and 0.5 mM putrescine (Put) and green plantlet derived from anther culture (C) of rice line 21.

Conclusion

An accumulation of glycine betaine in Homjan rice (HJ) was an effective defense in osmoregulation system, which played a role in cell stabilization in term of pigments, membrane and proteins. HJ rice was identified as a salt-tolerant genetic resource for breeding program. A rapid salt-tolerant screening was developed using multivariate cluster analysis. The rice line 21 was already classified as salt tolerant progeny to produce a pure line population using anther culture. Green plantlets derived from anther culture are successfully produced.

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