Indian Journal of Agricultural Sciences **83** (11): 1221–6, November 2013/Article

In vitro selection for NaCl salt tolerance in aromatic rice (*Oryza sativa*) genotypes

M HASAN[1] and R H SARKER[2]

Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur 1706, Bangladesh

Received: 9 January 2012; Revised accepted: 1 May 2013

ABSTRACT

Dehusked seeds of eight aromatic rice (*Oryza sativa* L.) varieties as parents and their F1 and F2 generations were placed on MS media supplemented with NaCl salt at four different levels (0%, 0.2%, 0.4% and 0.6%) to observe the callus induction and plant regeneration responses. For callus induction MS medium was supplemented with 2 mg/L 2,4 D while for plant regeneration MS medium was supplemented with 1 mg/L NAA and BAP. All the genotypes showed significant variation in callus induction and plant regeneration responses. Callus induction and plant regeneration percentage decreased with the increase of salt doses. While checking the response of parents under NaCl stress, the highest callus induction was found in Jirabhog and Badshahbhog (70%). Uknimadhu was best in plant regeneration (37.5%). For F_1 generation, Uknimadhu × Chinishakkhor showed the highest callus induction response (70). Uknimadhu × BR 5 was the best performer (27.5%) in plant regeneration response. For F_2 generation, Kalijira × Chinishakkhor showed the highest callus induction response (32.5%). For plant regeneration, Uknimadhu ×Kataribhog (10%) was the best. It was concluded that salt tolerant aromatic rice varieties can be obtained employing salt stress in in vitro condition..

Key words: Aromatic rice, Callus induction, Regeneration, Salt stress, Somaclonal variation

Rice (*Oryza sativa* L.) is one of the most important staple crops which provides the source of carbohydrates for more than half of the world's population (Tyagi *et al*. 2004). The world's projected demand of rice by 2020 is 880 million tonnes in proportion to the increasing population (Anbazhagan *et al*. 2009). Although with the increasing population and decreasing land availability, agriculture is very much suffering from severe damage of biotic and abiotic stresses. Biotic stress and abiotic stress breeding are essential ways to combat yield reduction. Scientists around the world are putting in their best efforts to produce varieties and hybrids with improved heterosis under stress affected environments (Sankar et al. 2011).

Conventional breeding is essential to improve rice but progress is slow due to different barriers (Wang *et al*. 2005). According to Shavindra *et al*. (2005), these challenges can be overcome by using biotechnological tools as a result of improved stress resistance with a high stable yield potential and good grain quality. Having mentioned this, rice is a salt sensitive plant, whereby the growth and productivity are unfavorably affected by salt stress, which prevents the crop from reaching its full genetic potential as well as limits its yield (Blumwald and Grover 2006). Hence, the use of *in vitro* technique has been employed in rice tissue culture in order to understand the mechanism of salinity stress in the plant. Recent advances in *in vitro* plant cell, tissue and organ culture as an additional method for crop improvement has been well augmented. Successful application of *in vitro* techniques of somatic tissue offers a wide scope of achieving somaclonal variants in regenerated plants from the rice calli.

Saline soils represent a massive and increasing challenge to the plant breeder and agronomist in many parts of the world. Crop plants selected for increased tolerance to saline environments should permit the use of those salt-affected soils. Traditional breeding strategies might be supplemented by the production of plants with improved salt tolerance through selection of the salt tolerance by *in vitro* culture and subsequent regeneration of such cells in the presence of salt to derive plants with improved salt tolerance.

Soil salinity is one of the important factors limiting the growth and productivity of rice in saline prone areas of the world (Lee *et al.* 2003). The effect of *in vitro* selection of rice cells with salt-tolerant phenotype for producing fertile plants with improved salt tolerance has been reported quite earlier by Winicov (1996). Initial salt stress in the medium has also

[1]Associate Professor, Department of Genetics and Plant Breeding (e mail: saikathu@yahoo.com), [2]Professor, Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh (e mail: rhsarker2000@yahoo.co.uk)

been reported to improve the frequency of somatic embryogenesis and plantlet regeneration (Lutts *et al.* 1999, Ping *et al.* 2006 ,Tariq *et al.* 2008). It is possible that such plantlets can be adapted to a saline environment resulting in enhanced crop production (Anitha and Reddy 1997).

Rice seed culture is an important tool for crop improvement program, which is widely used in variety development programs. It offers many advantages to rice breeders because of their shortened breeding periods and high efficiency in selecting useful recessive agronomic traits. Several local aromatic rice varieties are present in Bangladesh but their *in vitro* evaluation at different salinity levels has not yet been studied. Henceforth, the present study was undertaken to find out the *in vitro* response of different aromatic rice varieties and their F_1 and F_2 generations under NaCl salt stress.

MATERIALS AND METHODS

Eight Bangladeshi aromatic rice varieties and some cross combinations of their F_1 and F_2 generations were used in this study (Table 1).

Mature dehusked seeds were surface sterilized with 0.1% $HgCl_2$ for 20 minutes followed by 4-5 rinse in autoclaved distilled water to remove traces of $HgCl_2$. The seeds were then cultured on MS (Murashige and Skoog 1962) medium supplemented with 2 mg/L 2,4 D, 30 g/L sucrose, 10% agar and different levels of NaCl salt (0, 0.25, 0.45 and 0.65%) to initiate callus. After 2 to 3 weeks of inoculation calli were developed and transferred to MS regeneration medium supplemented with 1 mg/L NAA, 1 mg/L BAP, 30 g/L sucrose, 10% agar and above mentioned different levels of salts. Cultures were maintained in a growth chamber under 16 h photoperiod with a light intensity of 2000 lux under fluorescent tubes and proliferation of callus as well as frequency of plant regeneration were studied.

After 4 weeks of growth, the regenerated plantlets were washed under running tap water to remove agar and were transplanted to pots containing soil and cowdung in 2:1 ratio. Plantlets were covered with polythene bags. Inner sides of these bags were moistened with water to prevent desiccation.

Table 1 List of materials used in the experiment

Parents	F_1 and F_2 generations
Uknimadhu	Uknimadhu × Kataribhog
Kataribhog	Ukinimadhu × Kataribhog
Badshahbhog	Uknimadhu × Badshahbhog
BR 5	Ukinimadhu × BR 5
Chinishakkhor	Ukinimadhu × Chinishakkhor
Rajbhog	Uknimadhu × Rajbhog
Kalijira	Kalijira × Chinishakkhor
Jirabhog	Kalijira × Jirabhog
	Kalijira × Kataribhog
	Kalijra × BR 5
	Jirabhog × BR 5

After two days, polythene cover was perforated to expose the plants under a natural environment. Finally, the polythene bags were completely removed after 4-5 days. When the regenerated plants were fully established in the small pots, they were then transferred to larger pots for further growth and to get seeds from those regenerated plants.

The Analyses of Variance for different characters such as callus induction and plant regeneration were performed and the means compared by the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

High frequency of plant regeneration from callus of seed explants in salt supplemented media offers a feasible propagation method in rice which can be utilized for the improvement of salt tolerant varieties. In the present study, *in vitro* technique for callus induction and plant regeneration on NaCl salt supplemented media was established using seed of different indigenous Bangladeshi aromatic rice varieties and their F_1 and F_2 generation as explant. Effect of NaCl on callus induction and plant regeneration was also investigated. The results are elaborated based on the nature of morphogenetic response of genotypes, salt and salt concentrations and their interaction.

Effect of NaCl salt on callus induction and plant regeneration

Effect of NaCl salt on callus induction and plant regeneration is elaborated under the following titles.

Varietal/genotypic difference

The experiment was conducted to find out whether the genotypes show any differences in terms of callus induction and plant regeneration on the NaCl salt supplemented media. The mean performances of parents and F_1s and F_2s are summarized in Table 2.

Parents showed highly significant variations according to the Table 2. The highest callus induction was found in Jirabhog and Badshahbhog (70%) followed by BR 5 (67.5%). The lowest response was observed in Kataribhog (25%).

In terms of plant regeneration, the best performer was Uknimadhu (37.5 %), while the lowest response was observed in Rajbhog (2.5 %).

While checking the F_1s, Uknimadhu × Chinishakkhor showed the highest callus induction response (70%), while the lowest was found in Kalijira × BR 5 (5%).

Plant regeneration was highest in Uknimadhu × BR 5, while no plant regeneration was observed in Kalijira × Jirabhog.

In F_2, Kalijira × Chinishakkhor showed the highest callus induction (32.5%), while the lowest was found in Jirabhog × BR 5 (5%).

Highest plant regeneration was observed in Ukinimadhu × Kataribhog (10%). Jirabhog × BR 5 did not have any plant regeneration.

Table 2 Mean performance of parents and their F_1s and F_2s on callus induction and plant regeneration under NaCl stress

Parents	Characters		F_1	Characters		F_2	Characters	
	Callus induct. (%)	Plant reg. (%)		Callus induct. (%)	Plant reg. (%)		Callus induct. (%)	Plant reg. (%)
Uknimadhu (UM)	42.5 f	37.5 a	UM × KB	17.5 f	7.5 c	UM × KB	17.5 e	7.5 b
BR 5	67.5 b	25 c	UM × BR 5	60 b	27.5 a	UM × BR 5	25 c	7.5 b
Kalijira (KJ)	45 e	15 d	KJ × KB	12.5 h	5 d	KJ × KB	10 g	2.5 d
Kataribhog (KB)	*25 g*	5 f	KJ × CS	20 e	5 d	KJ × CS	32.5 a	5 c
Chinisakkhor (CS)	55 d	30 b	KJ × BR 5	*5 i*	2.5 e	KJ × BR5	12.5 f	2.5 d
Jirabhog (JB)	70 a	10 e	UM × CS	70 a	15 b	UM × CS	10 g	5 c
Badshahbhog (BB)	70 a	5 f	UM × KB	45 c	7.5 c	UM × KB	22.5 d	10 a
Rajbhog (RB)	60 c	2.5 g	UM × RB	25 d	7.5 c	UM × RB	27.5 b	5 c
			UM × BB	15 g	7.5 c	UM × BB	22.5 d	7.5 b
			KJ × JB	15 g	*0 f*	KJ × JB	17.5 e	2.5 d
			JB × BR 5	12.5 h	5 d	JB × BR 5	*5 h*	*0 e*

Table followed by the same letter in a column do not differ significantly by DMRT.

Based on the above findings, it was clear that all the genotypes showed significant variation in callus induction and plant regeneration responses under NaCl stress. Aditya and Baker (2005) found significant variation among four indigenous Bangladeshi aromatic rice varieties under NaCl stress.

Effect of NaCl salt concentrations on different genotypes

The interaction between the genotype and salt concentration was found statistically significant both for callus induction and plant regeneration as well which is shown in Table 3.

The highest callus induction was recorded in Kalijira, Chinishakkhor, Badshahbhog and Rajbhog (100%) on the control media (salt-free media). BR 5 occupied the second highest position with 90% callus induction response on the media supplemented with 0.2% NaCl salt. The lowest callus induction was recorded in Kalijira and Kataribhog (10%) on 0.6% salt supplemented media. It should be mentioned here that Kalijira had the highest response on control media, i.e. salt-free media, but with the increase of salt doses, the callus induction showed decreasing trend for this variety. This trend was similar for other varieties also with the increase of salt doses.

According to the Table 3, the highest plant regeneration was observed in Uknimadhu (70%) on the control media. No plant regeneration was observed in Kataribhog, Badshahbhog, Rajbhog at 0.2% NaCl salt concentration, Kalijira, Jirabhog, Badshahbhog, Rajbhog at 0.4% and Kalijira, Kataribhog, Jirabhog, Badshahbhog and Rajbhog at 0.6% NaCl salt concentration.

The interaction between the F_1s and salt concentrations was also found to be statistically significant for callus induction and plant regeneration which is shown in Table 4.

The highest callus induction was found in Uknimadhu × BR 5 and Uknimadhu × Chinishakkhor (90%) on the salt-free media (Table 4). It was also highest for Uknimadhu × BR 5, Uknimadhu × Chinishakkhor and Uknimadhu ×

Table 3 Callus induction and plant regeneration frequency of eight rice genotypes at different NaCl salt concentrations

Parents	Callus induction (%)				Plant regeneration (%)			
	Salt concentration (%)				Salt concentration (%)			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6
Ukinimadhu	70 d	50 f	30 h	20 I	70 a	50 c	30 e	0 h
BR 5	50 f	90 b	70 d	60 e	50 c	20 f	20 f	10 g
Kalijira	100 a	40 g	30 h	*10 j*	40 d	20 f	*0 h*	*0 h*
Kataribhog	40 g	30 h	20 i	*10 j*	20	*0 h*	*0 h*	*0 h*
Chinishakkhor	100 a	50 f	20 i	50 f	60 b	30 e	20 f	10 g
Jirabhog	80 c	70 d	80 c	50 f	30 e	10 g	*0 h*	*0 h*
Badshahbhog	100 a	70 d	60 e	50 f	20 f	*0 h*	*0 h*	*0 h*
Rajbhog	100 a	50 f	50 f	40 g	10 g	*0 h*	*0 h*	*0 h*

Table followed by the same letter in a column do not differ significantly by DMRT.

Table 4 Callus induction and plant regeneration frequency of F_1s at different NaCl salt concentrations

F_1	Callus induction (%)				Plant regeneration (%)			
	Salt concentration (%)				Salt concentration (%)			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6
UM × KB	50 d	10 h	10 h	*0 i*	30 c	*0 f*	*0 f*	*0 f*
UM × BR 5	90 a	90 a	50 d	10 h	70 a	30 c	10 e	*0 f*
KJ × KB	50 d	*0 i*	*0 i*	*0 i*	20 d	*0 f*	*0 f*	*0 f*
KJ × CS	60 c	20 g	*0 i*	*0 i*	20 d	*0 f*	*0 f*	*0 f*
KJ × BR 5	20 g	*0 i*	*0 i*	*0 i*	10 e	*0 f*	*0 f*	*0 f*
UM × CS	90 a	90 a	60 c	40 e	40 b	10 e	10 e	*0 f*
UM × KB	70 b	90 a	10 h	10 h	20 d	10 e	*0 f*	*0 f*
UM × RB	50 d	20 g	20 g	10 h	20 d	10 e	*0 f*	*0 f*
UM × BB	50 d	10 h	*0 i*	*0 i*	30 c	*0 f*	*0 f*	*0 f*
KJ × JB	30 f	20 g	10 h	*0 i*	*0 f*	*0 f*	*0 f*	*0 f*
JB × BR 5	40 e	10 h	*0 i*	*0 i*	20 d	*0 f*	*0 f*	*0 f*

Table followed by the same letter in a column do not differ significantly by DMRT.

Kataribhog on the media supplemented with 0.2% salt. No callus was formed in Kalijira × Kataribhog on 0.2, 0.4, 0.6% NaCl salt-supplemented media, Kalijira × Chinishakkhor on 0.4 and 0.6%, and Kalijira × BR 5 on 0.2, 0.4, 0.6% and Uknimadhi × Kataribhog on 0.6% NaCl salt-supplemented media.

The highest plant regeneration was recorded in Uknimadhu × BR 5. Kalijira × Jirabhog did not have any plant regeneration even in salt free media. No plant regeneration was observed in 0.6% salt supplemented media.

The interaction between the F_2s and salt concentrations were also found statistically significant for callus induction and plant regeneration which is shown in Table 5.

The highest callus induction was found in Uknimadhu × Rajbhog and Uknimadhu Badshahbhog (70%) on the salt-free media. No callus was formed in Uknimadhu × Kataribhog and Uknimadhu × BR 5 on 0.6% salt supplemented media, Kalijira × Kataribhog on 0.2 and 0.6%, Kalijira × BR5, Uknimadhu × Kataribhog and Kalijira 5 Jirabhog with 0.4 and 0.6%, Uknimadhu × Chinishakkhor and Jirabhog × BR5 on 0.2, 0.4 and 0.6% NaCl salt-supplemented media.

The highest plant regeneration was observed in Uknimadhu × Kataribhog on the salt-free media. No plant regeneration was recorded when the media was supplemented with 0.4% and 0.6% NaCl concentration.

The results of Table 3 to 5 have the similar findings with the experiment of Dang and Lang (2003), Shankhdhar *et al*. (2000) and Priya *et al*. (2011). They found that in a low concentration of salt-supplemented media, all tested cultivars developed normally, but the cultivars which responded poorly

Table 5 Callus induction and plant regeneration frequency of F_2s at different NaCl salt concentrations

F_1	Callus induction (%)				Plant regeneration (%)			
	Salt concentration (%)				Salt concentration (%)			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6
UM × KB	40 d	20 f	10 g	*0 h*	20 b	10 c	*0 d*	*0 d*
UM × BR 5	50 c	30 e	20 f	*0 h*	20 b	10 c	*0 d*	*0 d*
KJ × KB	30 e	*0 h*	10 g	*0 h*	10 c	*0 d*	*0 d*	*0 d*
KJ × CS	60 b	10 g	10 g	50 c	20 b	*0 d*	*0 d*	*0 d*
KJ × BR 5	30 e	20 f	*0 h*	*0 h*	10 c	*0 d*	*0 d*	*0 d*
UM × CS	40 d	*0 h*	*0 h*	*0 h*	20 b	*0 d*	*0 d*	*0 d*
UM × KB	50 c	40 d	*0 h*	*0 h*	30 a	10 c	*0 d*	*0 d*
UM × RB	70 a	20 f	10 g	10 g	20 b	*0 d*	*0 d*	*0 d*
UM × BB	70 a	20 f	*0 h*	*0 h*	20 b	10 c	*0 d*	*0 d*
KJ × JB	50 c	20 f	*0 h*	*0 h*	10 c	*0 d*	*0 d*	*0 d*
JB × BR 5	20 f	*0 h*	*0 h*	*0 h*	*0 d*	*0 d*	*0 d*	*0 d*

Table followed by the same letter in a column do not differ significantly by DMRT.

Table 6 Mean effect of NaCl salt concentrations on callus induction and plant regeneration in parents and F_1s and F_2s

Treatment (%)	Parents		F1		F2	
	Callus induction (%)	Plant regeneration (%)	Callus induction (%)	Plant regeneration (%)	Callus induction (%)	Plant regeneration (%)
0	80 a	37.5 a	54.55 a	20.55 a	46.36 a	16.36 a
0.2	56.25 b	16.25 b	32.73 b	5.46 b	16.36 b	3.64 b
0.4	45 c	8.75 c	14.55 c	1.82 c	5.46 c	*0 c*
0.6	*36.25 d*	*2.5 d*	*6.36 d*	*0 d*	5.46 c	*0 c*

Table followed by the same letter in a column do not differ significantly by DMRT.

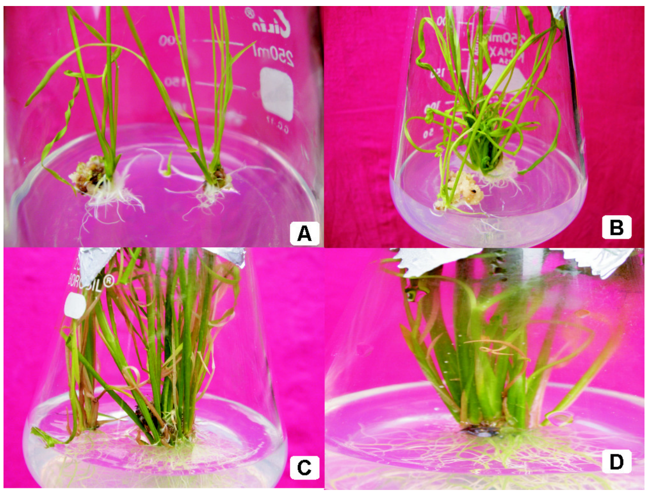

Fig 1 (A-C) Plant regeneration of different varieties, A. Chinisakkhor at 0.6% NaCl, B. Uknimadhu at 0.4% NaCl, C. Uknimadhu × BR 5 (F_1) at 0.4% NaCl, D. Uknimadhu × Kataribhog (F_2) at 0.2% NaCl

were from the media supplemented with high levels of salt and gradual reduction in regeneration was observed with increasing salt concentrations. This obviously suggests that salt concentration inhibits callus initiation and plant regeneration as well.

Effect of NaCl salt concentrations on callus induction and plant regeneration

Different concentrations of salt showed significant variations for callus induction and plant regeneration of different aromatic rice genotypes (Table 6).

Callus induction and plant regeneration was highest in control treatment while the lowest one was found with the highest dose of salt concentration (0.6%) for both the parents and their F_1 and F_2 generations as well.

According to Table 6, it is clear that different salt concentrations had paramount influence in callus induction and plant regeneration as well. On an average, all the parameters were arrested at high level of salt concentration (0.6%) and the magnitude of two parameters was maximum at 0% salt supplemented media. The results are in agreement with the experiment of Pushpam and Rangasamy (2000), where they found that the callus induction and plant regeneration percentage decreased with the increase of salt doses. They also reasoned that decline in growth of callus in the NaCl environment was due to diversion of some quantum of energy for growth and metabolism.

In conclusion, it can be said that salt-tolerant somaclonal variants can be obtained in rice and the progenies of some of the Bangladeshi aromatic rice varieties can also be utilized in developing salt tolerant rice varieties.

ACKNOWLEDGEMENT

The first author is grateful to the University Grants Commission of Bangladesh and University of Dhaka, Bangladesh for the support to carry out the research under the Post-Doctoral fellowship.

REFERENCES

Aditya T L and Baker D A. 2005. The effect of continuous NaCl stress on the restoration of high callus induction frequency, regeneration potential and *in vitro* growth of somaclones of four Bangladeshi indica rice genotypes. *Oryza* **42**: 103–8.

Anbazhagan M, Rajendran R, Kalpana M, Natarajan V and Panneerselvam R. 2009. Agrobacterium mediated Transformation of rice, var. Pusa Basmati-1. *J Ecol Biotechnol* **1**: 7–11.

Anitha S K and Reddy G M. 1997. Anther culture studies and selection of salt tolerant cell lines from indica rice. *Biological Sciences* **63**: 99–106.

Blumwald E A and Grover A. 2006. *Salt Tolerance in Plant Biotechnology: Current and Future Uses of Genetically Modified Crops*, pp 206–24. John Wiley and Sons Ltd., UK.

Dang M T and Lang N T. 2003. *In vitro* selection for salt tolerance in rice. *Omonrice* **11**: 68–73.

Lee K, Jeon H and Kim M. 2003. Optimization of a mature embryo-based in vitro culture system for high-frequency somatic embryogenic callus induction and plant regeneration from japonica rice cultivars. *Plant Cell, Tissue and Organ Culture* **71**: 237–44.

Lutts S J, Bouharmont J and Kinet J M. 1999. Physiological characterisation of salt-resistant rice (*Oryza sativa*) somaclones. *Australian Journal of Botony* **47**: 835–49.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* **15**: 473–97.

Ping W, Gang W and Jing J. 2006. NaCl tolerance of different genotypes in rice under tissue culture. *Journal of Jilin Agricultural University* **28**: 601–05.

Priya A M, Pandian S K and M. Ramesh M. 2011. Effect of NaCl on *in vitro* plant regeneration from embryogenic callus cultures of 'cv IR 64' indica rice (*Oryza sativa* L.). *African Journal of Biotechnology* **10**: 6947–53.

Pushpam R and Rangasamy S R S. 2000. *In vitro* response of rice genotypes to salt stress. *Madras Agricultural Journal* **87**: 694–97.

Sankar P D, Saleh M A and Selvaraj C I. 2011. Rice breeding for salt tolerance. *Research in Biotechnology* **2**: 1–10.

Shankhdhar D, Shankhdhar S C, Mani S C and Pant R C. 2000. *In vitro* selection for salt tolerance in rice. *Biologia Plantarum* **43**: 477–80.

Shavindra J and Amitabh M. 2005. Advances in rice biotechnology in the new millennium. *Plant Biotechnology Journal* **3**: 275–307.

Tariq M, Ali G, Hadi F, Ahmad S, Ali N and Shah A A. 2008. Callus induction and *in vitro* plant regeneration of rice (*Oryza sativa* L.) under various conditions. *Pakistan Journal Biological Science* **11**: 255–59.

Tyagi A K, Khurana J P, Khurana P, Raghuvanshi S, Gaur A, Kapur A, Gupta V, Kumar D, Ravi V, Khurana S and Sharma S. 2004. Structural and functional analysis of rice genome. *Journal of Genetics* **83**: 79–99.

Wang Y, Xue Y and Jiayang L J. 2005. Toward molecular breeding and improvement of rice in China. *Trends in Plant Science* **10**: 610–14.

Winicov I. 1996. Characterization of rice (*Oryza sativa* L.) plants regenerated from salt-tolerant cell lines. *Plant Science* **113**: 105–11.