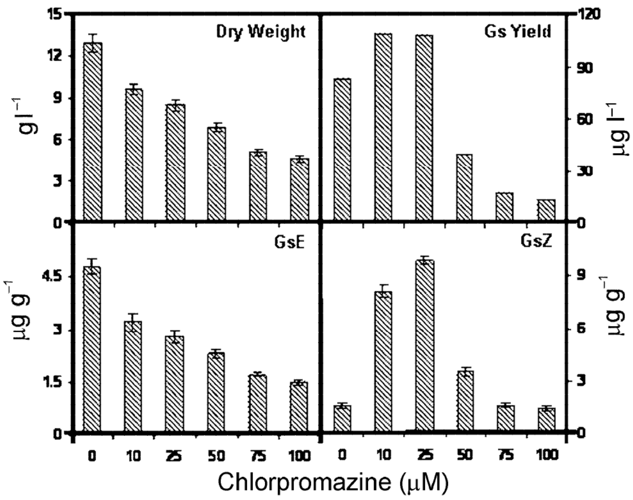

Figure 3. Effect of chlorpromazine on the growth and guggulsterone production in cell cultures of *C. wightii* grown in 250 ml flasks containing 100 ml MS medium. The cultures were harvested at 15 days.

total alkaloid excretion to the culture medium increased to 90% with 1 µM EGTA[10]. In contrast, EGTA inhibited the production of phytoalexins induced by an elicitor in *Allium cepa* cells[16] and sanguinarine production in *S. canadensis*[9]. Such increase in production of a secondary metabolite (Gs) has not been recorded earlier with EGTA in cell suspension cultures. A change in cytosolic Ca^{2+} concentration represents the signal which, directly or via Ca^{2+}-binding proteins, regulates the activities of respective target enzymes[10]. It may be inferred that instead of Ca^{2+} channels, Ca^{2+}-dependent enzymes are affected by Ca^{2+} deprivation, which results in enhanced Gs accumulation.

1. Ramawat, K. G., Mathur, M., Dass, S. and Suthar, S., *Bioactive Molecules and Medicinal Plants* (eds Ramawat, K. G. and Merillon, J. M.), Springer-Verlag, Heidelberg, 2008, pp. 101–121.
2. Urizar, N. L. *et al.*, *Science*, 2002, **269**, 1703–1706.
3. Ramawat, K. G. and Mathur, M., *Biotechnology Secondary Metabolites* (eds Ramawat, K. G. and Merillon, J. M.), Science Publishers, Enfield, USA, 2007, pp. 59–101.
4. Mathur, M., Jain, A. K., Dass, S. and Ramawat, K. G., *Indian J. Biotechnol.*, 2007, **6**, 525–531.
5. Mathur, M. and Ramawat, K. G., *Plant Biotechnol. Rep.*, 2008, **2**, 133–136.
6. Suri, S. S. and Ramawat, K. G., *Ann. Bot.*, 1997, **79**, 371–374.
7. Reddy, A. S. N., *Plant Sci.*, 2001, **160**, 381–404.
8. Merillon, J. M., Liu, D., Huguet, F., Chenieux, J. C. and Rideau, M., *Plant Physiol. Biochem.*, 1991, **29**, 289–296.
9. Mahady, G. B. and Beecher, C. W. W., *Phytochemistry*, 1994, **37**, 415–419.
10. Moreno-Valenzuela, O. A., Minero-Garcia, Y. and Chan, W., *Biotechnol. Lett.*, 2003, **25**, 1345–1349.
11. Mathur, M. and Ramawat, K. G., *Biotechnol. Lett.*, 2007, **29**, 979–982.
12. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
13. Kumar, S., Suri, S. S., Sonie, K. C. and Ramawat, K. G., *Indian J. Exp. Biol.*, 2003, **41**, 69–77.
14. Tanwar, Y. S., Mathur, M. and Ramawat, K. G., *Plant Growth Regul.*, 2007, **51**, 93–98.
15. Zhao, J., Hu, Q., Guo, Y.-Q. and Zhu, W.-H., *Plant Sci.*, 2001, **161**, 423–431.
16. Dmitriev, A., Djatsok, J. and Grodzinsky, D., *Plant Cell Rep.*, 1996, **15**, 945–948.

ACKNOWLEDGEMENTS. This work was supported by financial assistance from the DBT, New Delhi, and partially supported by DST–FIST programme for infrastructure development and UGC–DRS under special assistance programme for medicinal plant research to K.G.R.

Received 28 August 2008; revised accepted 6 March 2009

SUCHISMITA DASS
K. G. RAMAWAT*

Laboratory of Bio-Molecular Technology,
Department of Botany,
M.L. Sukhadia University,
Udaipur 313 001, India
**For correspondence.*
e-mail: kg_ramawat@yahoo.com

Development of first non-lodging and high-yielding rice cultures for saline kaipad paddy tracts of Kerala, India

Explosive increase in the world population, deterioration of arable land and availability of quality irrigation water are forcing crop production into more and more marginal environments facing abiotic stresses, thus limiting the adaptation and productivity of staple food crops[1]. In future, one cannot expect a major increase in land area available for cropping. At the same time, cultivated area is declining fast in most of the developing countries due to various reasons. It is estimated that half of the world's farms have been damaged by salt[2]. It has been estimated that about one billion hectares of the world's land is affected by salt, 60% of which is cultivated[3]. In the United States, it has been estimated that 28% of the irrigated area is salinized to a greater or lesser extent. China has salinity problems on 23% of its land, Pakistan 21%, India 11% and Mexico 10%. This is not a static situation and approximately 1.5 million hectares of irrigated land is salinized each year[4]. Improving salt tolerance of major crops is considered as the best practical approach to exploit the otherwise underutilized or completely unexploited salt-affected areas. In spite of a significant amount of research on the effect of salinity

Table 1. Yield of five promising rice cultures along with other selected breeding lines and check varieties in PYT and CYT in farmer's field

Cultures/breeding lines	Pedigree (parents)	Mean yield ($t\ ha^{-1}$) in	
		PYT	CYT
JK 70	Jaya[w] × Kuthiru[k]	7.6[a]	7.5[a]
JO 583	Jaya × Orkayama[k]	7.6[a]	6.9[a]
JO 532-1	Jaya × Orkayama	6.9[a]	6.9[a]
JO 345	Jaya × Orkayama	7.7[a]	6.8[a]
MK 22	Mahsuri[w] × Kuthiru	6.7[a]	6.5[a]
JK 74	Jaya × Kuthiru	6.4[a a]	5.8[aa]
OK 43	Orkayama × Kuthiru	5.7[aa]	5.7[aa]
JK 76	Jaya × Kuthiru	4.9[aaa]	4.9[aaa]
MK 146	Mahsuri × Kuthiru	2.2	2.3
JO 556	Jaya × Orkayama	1.4	1.2
JO 560-2-1	Jaya × Orkayama	1.1	1.1
JK 67	Jaya × Kuthiru	1.1	1.1
MK 162	Mahsuri × Kuthiru	1.1	1.1
JK 23	Jaya × Kuthiru	1.0	1.0
Vytilla 6 (high yielding pokkali check)		1.8	2.0
Kuthiru (local check)		2.1	2.2
Mean		4.1	3.9
CV %		16.3	15.2
CD (0.01)		0.90**	0.84**

[w]Wet-land variety susceptible to salinity; [k]Kaipad cultivar tolerant to salinity; [a]In a column, means followed by the same alphabet do not differ significantly from each other. PYT, Preliminary yield trial; CYT, Comparative yield trial.

Figure 1. Kaipad rice field at (***a***) vegetative and (***b***) harvest stages.

on plants, there has been little success in putting salt-resistant plants in the farmers' field[5].

As rice is one of the major food crops, development of new cultivars with enhanced salt stress-tolerance will undoubtedly have an important effect on global food production. During 2007, rice production of India and the world was 1435 and 6365 lakh tonnes respectively[6]. As soil salinity tolerance of rice is a complex trait of several physiological characters[7], research on the line of development of rice varieties tolerant to salinity is meagre. Further, production of organic rice is a necessity of the present era. Hence, development of high-yielding rice varieties, suitable for a salinity stress area which is naturally an organic production system like the 'kaipad' rice tract, is imperative in rice breeding.

Kaipad is a saline-prone natural organic rice production tract of North Kerala, India, like the pokkali tract of South Kerala. The kaipad system of rice cultivation is an integrated organic farming system in which rice cultivation and aquaculture go together in coastal brackish-water marshes, which are rich in organic matter. The soil type is saline hydromorphic[8]. The network of backwaters and estuaries serves as an inlet of sea water and causes salinity in the area. Rice farming is carried out in a purely natural way in kaipad relying on the monsoon and the sea tides (Figure 1). Single-crop of rice is cultivated on mounds in a low-to-medium saline phase of the production cycle during June–October. In June, after the southwest monsoon, germinated seeds of local saline-tolerant cultivars are sown on the flattened tops of the mounds. In a month, the mounds are dismantled and the seedlings in clefts are dispersed around the flattened mounds. There is no need of any cultural operations till the harvest. Harvesting takes place by the end of October. This is followed by traditional fishing, during the high saline phase, from November to April. Neither chemical fertilizers nor plant protection chemicals are used in rice, fish, or shrimp farming. The tidal flows make the fields highly fertile through a symbiotic relationship between the rice crop and prawn, shrimp, fish, etc. The seedlings of fish, shrimp, prawn, etc. which swim in from the sea and the backwaters after the rice harvest, feed on the leftovers of the harvested crop. The rice crop draws nutrients from the excrement and other remnants of these sea creatures. Also, there is no purposeful removal of weeds as practised in modern rice-farming systems. Further, diversity of flora and fauna is rich when compared to modern rice-farming system. In addition, the fertility of the field is increased due to left-over rice stubbles and post-harvest vegetations, including submerged macrophytes.

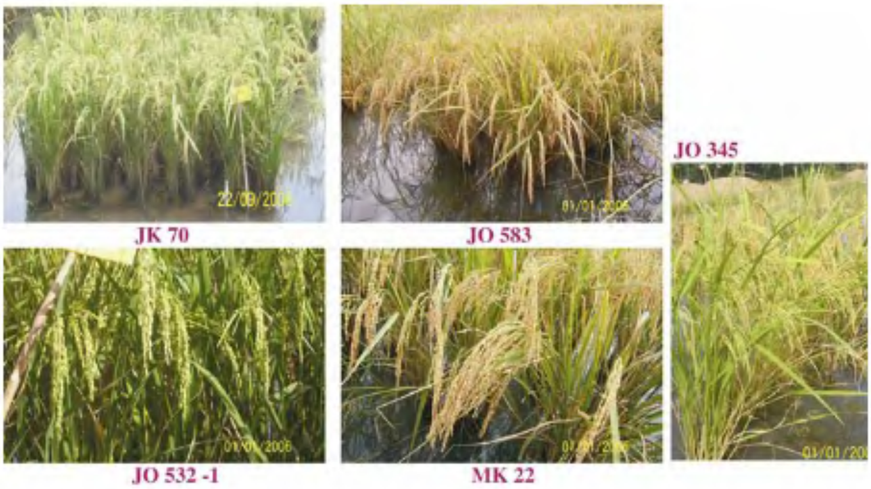

Figure 2. The first non-lodging and high yielding rice cultures for saline kaipad paddy tracts.

Forty years ago, about 2500 ha of kaipad rice fields existed in the Kannur District, Kerala. But now it has been restricted to about 600 ha. Most of the kaipad fields either lie barren or produce low yields. At present, traditional cultivars, namely 'Kuthiru', 'Orkayama', 'Mundon', 'Kandorkutty', 'Orpandy', 'Odiyan' and 'Orissa', tolerant to low and medium salinity are cultivated in various kaipad fields in Kerala. The average rice yield of these local cultivars is about 2000 kg ha^{-1}, making rice cultivation in this region unprofitable. Lack of realization of the potential of high-yielding rice varieties to this rainfed, shallow lowland is the major reason for the low productivity and shrinkage of kaipad fields. The traditional cultivars are susceptible to lodging, because of the poor culm strength and excessive culm length, with poor grain qualities like awn on grains, long bold and heavy shattering of grains. Panicles of these cultivars are long but less in the number of grains. However, these cultivars are resistant to all pests and diseases in natural field conditions of kaipad and the cooked rice is delicious. The high-yielding saline-resistant pokkali varieties do not perform well in kaipad saline tracts. This may be due to the difference in the physico-chemical properties of both soils. The soil pH throughout the depth of soil profiles of kaipad is slightly acidic, whereas that of pokkali is slightly alkaline[8].

High-yielding rice varieties suitable to kaipad rice tracts have not been developed yet. Development of varieties, having high yield, medium height, tolerance to salinity and lodging, favourable grain and cooking qualities, non-shattering and awnless, is a demand of the farmers of this area for a long time. Sustained and systematic research efforts have resulted in the development of high-yielding rice cultures. As there are increasing demands for organic rice across the world market, development of this type of rice varieties, suited to organic production system, is the need of the hour.

The efficiency of breeding for salt tolerance was perceived to be low because of the evident genetic complexity of the trait, large genotype × environment interactions, and the problem of controlling relevant environmental variables during field-based selection[5]. In order to improve the suitability of the varieties produced to specific local farming situations, the new approach recommends farmer participatory varietal selection for salt-tolerant breeding programmes[9]. Hence, a farmer participatory breeding programme was carried out to develop high-yielding varieties suited to kaipad rice field.

Participatory plant breeding (PPB) is the latest strategy in the area of plant breeding to integrate end-user-based participatory approach[10]. PPB is based on a set of methods that involve close farmer–researcher collaboration to bring about plant genetic improvement within a crop. It is expected to produce more benefits than the traditional global breeding model in situations where a highly centralized approach is inappropriate. PPB methods are designed to incorporate the perspective of the farmers, usually by inviting them to participate in a variety of evaluation activities, realizing the fact that modern varieties developed for favourable production conditions have not always diffused readily into marginal environments. By involving farmers in the genetic improvement process, plant breeding programmes will be able to produce better varieties that will be adopted more widely and generate greater benefits on aggregate. PPB provides a means of assessing so-called subjective traits. In food crops these include taste, aroma, appearance, texture and other characteristics that determine the suitability of a particular variety for culinary use. These traits are difficult to measure quantitatively because they are a function of human perceptions.

Inter-varietal hybridization was carried out between varieties, such as 'Jaya' and 'Mahsuri', which are under cultivation in the proximity of the kaipad fields where there is a slight intrusion of salinity, and the popular saline-tolerant traditional cultivars of kaipad, such as 'Kuthiru' and 'Orkayama'. The F_1 generation of six cross combinations was raised in pots. Succeeding filial generations were raised as on-farm trials in the kaipad fields, with the participation of farmers during different stages of growth for selection of promising genotypes suitable to the saline flooded areas. Six thousand two hundred and ninety-two F_2 progenies were raised in the kaipad fields having slight salinity (2 dS m^{-1}), adopting organic rice farming practices. Only 1028 progenies survived in the slight salinity. Single plant pedigree selection was followed in F_2 generation. All the F_2 progenies that survived in the slight saline condition were carried forward to the F_3 generation in kaipad fields. From the F_3 generation onwards, all advanced filial generations were evaluated directly in the problem area having medium salinity. One hundred and nineteen progenies were found promising in the F_4 generation. In the F_5 generation, 14 stabilized breeding lines, which showed high yield in F_3 and F_4 generations, were evaluated in replicated yield trials along with the local check (Kuthiru) and high-yielding pokkali check (Vytilla 6) during the kharif season of 2007. The design of yield trials was RBD with three replications. Pests (gall midge, stem borer, leaf roller, whorl maggot and case worm) and diseases (sheath blight, neck blast, BLB and brown spot) scoring was done under natural field conditions at kaipad and also under artificial infection in wet-land condition at the Regional Agricultural

Table 2. Plant characteristics of the first five high-yielding kaipad rice cultures in comparison with their parents, kaipad check and pokkali check

	Cultures					Parents/checks				
Characteristics	JK 70	JO 583	JO 532-1	JO 345	MK 22	Jaya (parent)	Mahsuri (parent)	Orkayama (parent)	Kuthiru (local check and parent)	Vytilla 6 (*Pokkali* check)
Yield traits										
Grain yield (t ha^{-1})	7.45	6.9	6.85	6.75	6.54	2.1	1.86	2.25	2.15	2.02
Straw yield (t ha^{-1})	13.7	14.7	9.9	8	10.2	7	10.5	13.8	6.5	2.9
Harvest index	0.35	0.32	0.37	0.49	0.39	0.23	0.15	0.14	0.25	0.4
Yield increase over local check (%)	246.5	220.9	218.6	214	204.2	NA	NA	NA	NA	NA
Yield increase over pokkali check (%)	268.8	241.6	239.1	234.2	223.8	NA	NA	NA	NA	NA
Plant height (cm)	101.1	116	110.7	132	110.8	85.3	118.1	141.8	142.3	83.3
Nature of culm	Strong and sturdy	Strong and sturdy	Strong and sturdy	Strong and sturdy	Strong and sturdy	Strong and sturdy	Weak stem	Weak stem	Weak stem	Sturdy
Duration (days)	130–137	137–149	135–143	137–149	137–149	130	125	137	130	133
Lodging habit	Non-lodging	Non-lodging	Non-lodging	Non-lodging	Non-lodging	Non-lodging	Slightly lodging	Lodging	Lodging	Non-lodging
Tillers $plant^{-1}$ (no.)	18.9	13.9	15.4	20.8	16.3	3	8.4	13.5	7.7	12.7
Panicles $plant^{-1}$ (no.)	21.3	19.3	16.1	23.5	18.3	2	8.1	15.9	10.6	17.2
Panicle length (cm)	25.5	27.8	27.9	29.4	29.3	22.9	24	28.6	27.1	23.7
Grains $panicle^{-1}$ (no.)	116.6	167.9	175.7	283	289.3	101.8	126.8	73.9	90.7	122.2
1000 grain weight (g)	28.3	27.2	29.8	25.6	26	35.7	18.7	32.6	31.3	27
Quality traits										
Grain-shattering	Non-shattering	Non-shattering	Non-shattering	Non-shattering	Non-shattering	Non-shattering	Shattering	Shattering	Shattering	Non-shattering
Awn	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent
Hulling (%)	79.1	78.5	77.8	77.5	75.9	78.3	75.9	80.1	81.1	79.8
Milling (%)	76.9	76.8	75.6	75.3	74.3	67.5	71.3	75.1	74.8	74.3
Grain length (mm)	8.3	7.2	8.4	8.1	8.4	9.2	7.97	8.9	8.7	8.6
Grain breadth (mm)	3	2.9	3.2	3	3	3.2	2.75	3.2	3.3	3
Length–breadth ratio	2.76	2.49	2.63	2.7	2.8	2.9	2.91	2.78	2.63	2.86
Kernel colour	Red	Red	White	Red	Red	White	White	Red	Red	Red
Volume expansion ratio	3.2	4.3	2.8	3.3	2	2.3	2.8	2.8	3.5	2.8
Water uptake (ml)	1.6	2.1	1.7	1.9	2.6	0.6	0.54	1.68	1.69	1.7
Alkali spreading value	4.3	4	4.3	4.3	5	2.7	4	4.3	4	5
Amylose content (%)	26.4	26.5	23.5	29	29.8	23.8	26.5	23.64	25	29.7
Specific features of new cultures	The apicule colour is purple at flowering stage and turns straw colour on maturity. The base of the culm is purple at seedling stage. Thick, dark green erect leaves even at the time of harvest	Exhibits high multiple branching of productive tillers	Golden-coloured grains. Thick, green, erect leaves even at the time of harvest	Thick, dark green, erect leaves even at the time of harvest	Thick, dark green, erect leaves even at the time of harvest. Green-coloured panicle rachis even after harvest	–	–	–	–	–

Research Station, Pattambi, Kerala, India. Standard evaluation system for rice[11] was used for evaluating and for describing the cultures.

In the preliminary and comparative trials, the yield of eight cultures was found to be significantly higher than that of local and pokkali checks (Table 1). Among these eight cultures, the top ranking five cultures (Figure 2) which were statistically on par in yield, were considered for immediate follow-up action. The pedigree of these cultures is given in Table 1. The main plant characteristics were compared with those of their parents, local and pokkali checks (Table 2). These five cultures were high yielding (6.5–7.5 t ha^{-1}) and tolerant to medium salinity (8 dS m^{-1}). Further, unlike 'Kuthiru', these cultures had intermediate plant stature (101–132 cm), with strong and sturdy culm. Therefore, these cultures were resistant to lodging. These cultures were found to have more grain and straw yield and higher harvest index than that of 'Kuthiru'. These cultures were found to have medium duration, and grains non-shattering and awnless. They were resistant to all kinds of pests and diseases at the kaipad field condition. In artificial inoculation, they were invariably found to be resistant and moderately resistant to many pests and diseases. Besides their proven yield potential, pest and disease resistance and other preferable characters to saline and flooded areas, they possessed desirable grain qualities, better taste and more acceptable appearance of cooked rice, appealing to both consumers and millers. Most of the cooking qualities of all the cultures were on par with traditional cultivar 'Kuthiru' and better than the pokkali check 'Vytilla 6'. The culture JO 583 exhibited excellent cooking quality with volume of cooked rice 21.4% more than that of local check and 51.8% more than that of pokkali check. Four cultures were with red kernel colour, a trait much preferred by the people of Kerala. The culture JO 532-1 had golden-coloured grains and white kernel colour, the characters more preferred in the international market. All these cultures had higher 1000 grain weight, more number of grains $panicle^{-1}$ than parents and checks, with maximum number for the cultures JO 345 and MK 22, with very long panicle. In addition to the resistant nature of all cultures to flooded condition, the cultures JK 70, JO 532-1, JO 583 and JO 345 survived the 40 days flooded condition which occurred two days after germination during a natural calamity in the kharif season of 2007.

Morphologically each culture has its own peculiarities. The apicule colour of culture JK 70 was purple at flowering stage and turned straw colour on maturity. The base of the culm was purple at seedling stage. JO 532-1 had golden-coloured grains. The culture MK 22 possessed green-coloured panicle rachis even after harvest. The cultures JK 70, JO 532-1, JO 345 and MK 22 had thick, dark green, erect leaves even at the time of harvest and their plant stature was attractive. Culture JO 583 exhibited high multiple branching of productive tillers.

The five newly developed cultures have high yield, medium height, tolerance to salinity and lodging, favourable grain and cooking qualities, for the saline-prone kaipad paddy tracts of Kerala. These cultures have a wide genetic base, because one of the parents is a local cultivar having abiotic and biotic stress resistance, and preferable cooking qualities. Till now, there has been no high-yielding rice variety to kaipad. These five cultures will provide varietal diversity to the salt stress-prone kaipad field.

A major reason for farmers from the kaipad area to move away from rice cultivation has been unfavourable characters of locally available cultivars. The newly developed rice cultures have all the favourable characters like tolerance to medium salinity, non-lodging, intermediate plant type, high yield, acceptable grain qualities, and resistance to all kinds of pests and diseases at natural kaipad field condition. These cultures can tolerate flooded condition even immediately after sprouting and at early seedling stages. As the major part of the experiment was conducted in the farmer's field with the participation of farmers in the selection programme, they have been convinced about the yield potential and suitability of the new cultures to kaipad saline-flooded conditions. Hence, there is great demand from the farmers for the seeds of the newly developed rice cultures. These cultures are now in the pre-release stage and will be the ideal varieties for kaipad. Development of these new rice cultures helped in transforming the vast kaipad area into an arable and highly productive farming land. In future, it could increase the production and export of organic red rice from Kerala, thus helping the poor and marginal farmers of the state.

1. Sharma, S. K. and Goyal, S. S., In *Crop Production in Saline Environments: Global and Integrative Perspectives* (eds Sham, S. *et al.*), Food Products Press, An imprint of The Haworth Press, Inc., 2003, pp. 387–407.
2. Pearse, F., *New Sci.*, 1987, **11**, 53–56.
3. Epstein, E., Norlyn, J. D., Rush, D. W., Kingsbury, R. W. and Kelley, D. B., *Science*, 1980, **210**, 399–404.
4. Rains, D. W. and Goyal, S. S., In *Crop Production in Saline Environments*, 2003, pp. 1–8.
5. Flowers, T. J. and Yeo, A. R., *Aust. J. Plant Physiol.*, 1995, **22**, 875–884.
6. IRRI World Rice Statistics, IRRI, Manila, 2007, p. 1.
7. Flowers, T. J. and Yeo, A. R., Proceedings of the International Congress of Plant Physiology, New Delhi, 15–20 February, pp. 953–959.
8. Swarajyalakshmi, G., Gurumurthy, P. and Subbaiah, G. V., In *Crop Production in Saline Environments: Global and Integrative Perspectives* (eds Sham, S. *et al,*), Food Products Press, An imprint of The Haworth Press, Inc., 2003, pp. 247–275.
9. Bennet, J. and Khush, G. S., In *Crop Production in Saline Environments: Global and Integrative Perspectives* (eds Sham, S. *et al.*), Food Products Press, An imprint of The Haworth Press, Inc., 2003, pp. 11–65.
10. Morris, M. L. and Bellon, M. R., *Euphytica*, 2004, **136**, 21–35.
11. IRRI, Standard evaluation system for rice. International Rice Research Institute, Manila, The Philippines, 1988.

Received 14 June 2008; revised accepted 19 March 2009

T. VANAJA[1,*]
V. P. NEEMA[1]
K. P. MAMMOOTTY[1]
R. RAJESHKUMAR[1]
P. C. BALAKRISHNAN[2]
JAYAPRAKASH NAIK[2]
P. RAJI[3]

[1]*Pepper Research Station,*
Kerala Agricultural University,
Panniyur 670 142, India
[2]*Regional Agricultural Research Station,*
Kerala Agricultural University,
Pilicode 671 363, India
[3]*Regional Agricultural Research Station,*
Kerala Agricultural University,
Pattambi 679 306, India
**For correspondence.*
e-mail: vtaliyil@yahoo.com