Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 154-156

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Biochemical characterization of maintainers and restorer in rice

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Abstract
To elucidate the nature and extent of differentiation in maintainers and restorers of cytoplasmic male sterility system in rice and to investigate the indica and japonica type divergence of breeding lines. The experimental materials comprised of ten restorers and ten maintainers of wild abortive cytosterility system and two checks from indica and japonica backgrounds. The observations on apiculus hair length, germination speed, degree of resistance to low temperature and potassium chlorate, absorbance of phenol solution after soaking grains, phenol reaction of grain surface, localisation of phenoloxidation system and peroxidase isozymes pattern were recorded to ascertain the nature and extent of differentiation and divergence. Three replications were made for the former five characters. The inference was derived precisely with the help of mean squares, mean values, range, critical difference, taxonomic distance and similarity coefficient based dendrogram and peroxidase zymogram. Maintainers showed relatively greater range of variation for germination speed, degree of resistance to low temperature and potassium chlorate, same range of variation was noticed in maintainer restorers for the apiculus hair length and absorbance of phenol solution after soaking grains. Predominance of indica check like combination of short apiculus hairs, positive phenol reaction and greater degree of low temperature and potassium chlorate resistance was noticed in restorers. Five restorers while one maintainer exhibited indica check like characters combination. Promising restorers and maintainers should be produced separately by inter-crossing and subsequent selection in the course of their improvement where one pool of inter-crossing should be for restorers and another for maintainers. The findings of this study implied that the pools of promising restorers and maintainers should be produced separately by inter-crossing and subsequent selection in the course of their improvement where one pool of inter-crossing should be for restorers and another for maintainers. In this way, the genetic diversity retained between these two groups of breeding lines should provide for an enhanced level of heterosis when utilised in breeding programmes.

Keywords: Cytoplasmic male sterile, Heterosis, Maintainers, Restorers, Indica

Introduction
Indisputably, the quality of life of most of the people in our country has been enhanced through green revolution. The country not only attained self-sufficiency in food grain production in the eighties but also earned a place for it among the major food grain exporting countries in the nineties. During the last five decades, while the area under rice has increased only by one and half times, its production has increased more than four times and the productivity has increased three times (Mishra, 2004). Rice exports have grown steadily during the last decade. Recording a dramatic increase in rice production since the inception of high yielding, photoperiod insensitive and nitrogen responsive varieties into commercial cultivation, together with perfection of location specific agro-techniques, our country became the second largest producer of rice in the world. Among the rice trading countries of the world, our country now occupies the second position in rice exports. Keeping in view the annual population growth, the surplus production scenario, however, has no room for the complacency. With no further scope of expansion of area under the rice crop, the steady increase in production to feed the burgeoning population over the years has necessarily to come from increased productivity under depleting and diminishing resources. Vertical growth will being only option to sustain the current level of sufficiency. The challenge for the future is, therefore, not only to sustain current productivity gain but to increase productivity growth. The envisaged growth cannot be achieved through the saturation of untapped production reservoirs with high yielding varieties alone. Raising genetic yield ceiling is inevitable for ensuring continued yield growth in the high productivity areas. The further increase in productivity is a highly challenging task ahead and scientists are exploring the possibilities of raising the present ceiling to new heights. Genetic transformation promises to revolutionise rice improvement programme due to its capacity to mobilise useful genes from non-rice gene

pool to rice with least disruption to rice genome. Hybridisation between restorers/ restorers, restorers/maintainers, maintainers/restorers and cytoplasmic male sterile lines/restorers is attempted for the development and improvement of parental lines of three-line rice hybrids. Indica and japonica type genetic diversity is confirmed and retained between restorers and maintainers, it would serve to enhance the level of heterosis when utilised in breeding programmes with the following objectives:

1. To study the nature and extent of differentiation in maintainers and restorers of cytoplasmic male sterility system.
2. To survey the indica and japonica type divergence between restorers and maintainers

Materials and methods

This study was carried out in the laboratory of Department of Genetics at Rajendra Agricultural University, PUSA, Samastipur, Bihar.

The experimental materials of this study comprised of ten sterility maintainers(IR 58025B, IR 67684B, IR 68275B, IR 68280B, IR 68885B, IR 68888B, IR 68899B, IRC68902B, IR 69616B and IR 69626B) and ten fertility restorers of wild abortive cytoplasmic male sterility (IR 23352-7R, IR 42266-29-4-2-2-2R, IR 43342-10-1-1-3-3R, IR 59624-34-2-2R, IR 59669-93-1-3R, IR 60919-150-3-3-3-2R, IR 60997-16-2-3-2-2R, IR 62036-222-3-3-1-2R, IR 62037-12-1-2-2-2R and IR 63870-123-2-2-2-2R (hereafter designated as IR 23352R, IR42266R, IR43342R, IR59624R, IR59669R, IR60919R, IR60997R, IR62036R, IR62037R and IR63870R, respectively) and two checks, one each from indica (IR 36) and Japonica (Taipei 309).

Study of parameters

1. Apiculus hair length(mm):
2. Germination speed: Resistance to low temperature:
3. Shoot vigour(cm) = Length of shoot
4. Root vigour(cm)= Length of root
5. Coleoptile vigour (cm) = Length of coleoptile
6. Overall vigour(cm)= Length of root plus length of shoot plus length of coleoptile
7. Vigour index = Germination% X (Length of root plus length of shoot plus length of coleoptile)
8. Percentage decrease in vigour = _Vnt - Vlt _X100
   Vnt

Where, Vnt- Vigour at normal temperature
Vlt- Vigour at low temperature

9. Resistance to potassium chlorate:
10. Colour reaction with phenol:
11. Optical density of phenol solution:
12. Localisation of monophenol oxidation system:
13. Isozymes of peroxidase:

Statistical analysis

An average value for observations recorded on the five parameters. Namely, apiculus hair length, germination speed, resistance to potassium chlorate, optical density (absorbance) of phenol solution and resistance to low temperature on the basis of shoot vigour, root vigour, coleoptile vigour, overall vigour and vigour index at normal and low temperatures, percentage decrease in shoot vigour, root vigour, coleoptile vigour, overall vigour and vigour index, was computed for each entry in each of the three replications and then subjected to statistical analyses.

Result and discussion

An analysis of variance (Table-1) revealed significant genotypic differences for germination speed, degree of resistance to low temperature and potassium chlorate, apiculus hair length and absorbance of phenol solution after soaking grains. While maintainers showed relatively greater range of variation for the former three parameters, almost the same range of variation was noticed in maintainers and restorers for apiculus hair length and optical density of phenol solution. In general, maintainers had greater degree of resistance to low temperature and potassium chlorate but smaller apiculus hairs than in the restorers. A majority of the entries which had indica check like characters combination tended to cluster together in numerical taxonomic approach of classification based on apiculus hair length, germination speed, absorbance of phenol solution and degree of resistance to low temperature and potassium chlorate.

Table 1: Character combinations found in the genotypes of rice

Genotypes	Apiculus hair length	Germination speed	Resistance to low temperature	Resistance to potassium chlorate	Colour reaction of grain surface
IR 23352 R	0	0	1	0	0
IR 42266 R	0	1	0	0	0
IR 43342 R	0	1	0	1	0
IR 59624 R	0	1	0	0	0
IR 59669 R	0	0	0	0	0
IR 60919 R	0	0	1	0	0
IR 60997 R	0	0	1	0	1
IR 62036 R	0	0	1	1	0
IR 62037 R	0	0	0	0	0
IR 63870 R	0	0	0	0	0
IR 58025 B	0	1	1	1	0
IR 67684 B	0	0	1	1	0
IR 68275 B	0	0	1	0	0
IR 68280 B	0	0	1	1	1
IR 68885 B	0	1	0	0	0
IR 68888 B	0	0	1	0	0
IR 68899 B	0	0	1	0	0
IR 68902 B	0	1	0	1	0
IR 69616 B	0	0	1	0	0
IR 69626 B	0	0	1	0	0
IR 36	0	0	0	0	0
Taipei 309	1	0	1	1	1

0 & 1: Smaller and longer apiculus hairs, respectively
Lower and higher germination speed, respectively
Lesser and greater low temperature resistance, respectively
Lesser and greater KClo3 resistance, respectively
Positive and negative phenol reaction, respectively

Table 2: Presence and absence of monophenol oxidase in plant-parts of different genotypes of rice

Entry	Leaves	Nodes	Internodes	Rachis	Grains
IR 23352 R	-	-	-	-	+
IR 42266 R	-	-	-	-	+
IR 43342 R	-	-	-	-	+
IR 59624 R	-	-	-	-	+
IR 59669 R	-	-	-	-	+
IR 60919 R	-	-	-	-	+
IR 60997 R	-	-	-	-	-
IR 62036 R	-	-	-	-	+
IR 62037 R	-	-	-	-	+
IR 63870 R	-	-	-	-	+
IR 58025 B	-	-	-	-	+
IR 67684 B	-	-	-	-	+
IR 68275 B	-	-	-	-	+
IR 68280 B	-	-	-	-	-
IR 68885 B	-	-	-	-	+
IR 68888 B	-	-	-	-	+
IR 68899 B	-	-	-	-	+
IR 68902 B	-	-	-	-	+
IR 69616 B	-	-	-	-	+
IR 69626 B	-	-	-	-	+
IR 36	-	-	-	-	+
Taipei 309	-	-	-	-	-

+ & - : Presence and absence of monophenol oxidase in plant-parts

Table 2 visualised remarkable difference in peroxidase isozymes pattern of maintainers and restorers. Phenol oxidation system was localised in grains, but not in leaves, nodes, internodes and rachis. Only grains reacted positively with phenol solution indicating that monophenol oxidase was exclusively localised in grains.

Conclusions

The findings of this study implied that the pools of promising restorers and maintainers should be produced separately by inter-crossing and subsequent selection in the course of their improvement where one pool of inter-crossing should be for restorers and another for maintainers. In this way, the genetic diversity retained between these two groups of breeding lines should provide for an enhanced level of heterosis when utilised in breeding programmes.

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