Original Research Article

Foreground Selection of F₂ Segregants of the Cross ‘Rajendra Sweta X Swarna Sub1’

Ummay Hafsa¹*, S.P Singh¹ and Sweta Sinha²

¹Department of Genetics and Plant Breeding, ²Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Bhagalpur-813210, Bihar, India

*Corresponding author

A B S T R A C T

Submergence tolerant variety is a crucial requirement in a place like Bihar where floods are a common phenomenon and has the potential to destroy the crops. With this aim to develop a tolerant variety, the further advancement of generation of the cross between RajendraSweta and Swarna Sub 1 needs to be done. To fulfil this, the present research work was carried out at molecular biology laboratory and research farm area of Bihar Agricultural University, Sabour, Bhagalpur with the objective to screen the F₂segregants of RajendraSweta x Swarna Sub1 for Sub1 locus. Foreground selection was performed to identify the plants with Sub1 locus using Sub1BC2 marker which is linked to the Sub1 locus. Based on the foreground selection, it was found that fifty eight plants possessed RajendraSweta type allele, forty one plants were Swarna Sub1 type allele and eighty seven were heterozygote type allele (Both RajendraSweta and Swarna Sub1 type allele).

Keywords: F₂ segregants, Sub1 locus, Foreground selection, Swarna Sub1 type allele

Accepted: 10 October 2019
Available Online: 10 November 2019

Introduction

Rice is considered as one of the most important crops of India. It has a socio-economic importance in our lives due to the fact that in many Asian countries it’s used in sacred rituals. It has indubitably become a part of Asians souls and its importance is irreplaceable by any grain. Rice is usually grown in rainfed and lowland areas due to which its production is continually threatened by series of biotic and abiotic stress. Even though rice is being cultivated under flooded and irrigated condition, most of the rice varieties under cultivation are susceptible to flooding if the plants are submerged under water for more than sevendays¹. More than 16% of rice grown in lowlands is adversely
affected by floods every year worldwide. About 16.1 million ha of rainfed lowland rice are grown each year in India of which 5.2 million ha are periodically affected by submergence. Hence, developing submergence/flood tolerant rice genotypes will be useful in reducing yield loss in rice in these areas.

Marker assisted selection (MAS) has a great potential in the genetic enhancement of rice and several breeders used it as a tool for fastening the breeding by skipping several breeding cycles during the segregating generations. It is relatively more efficient than selection by phenotype alone. This can therefore be greatly used for characterization of the genotypes. Genotyping and phenotyping of the plant population enables us to identify the best segregants and is an effective tool for selection. Introggression of submergent tolerant gene into the local variety has evoked a new means of selection of the best and tolerant genotypes for a particular area.

As Bihar is a flood prone area, there is a great need of submergence tolerance ability in the local variety of Bihar (Rajendra Sweta). Rajendra Sweta is a high yielding (45-50 Qtls/ha), medium duration (125-130 days), semi-dwarf, medium slender grained variety which is prevalently grown in medium land ecology in most part of Bihar.

However, under the current climate change scenario the rice crop unpredictably suffers from frequent flash flood which is occurring mostly after 15th August in major portion of the state of Bihar which partially or fully damages the standing crop. On the other hand Swarna-Sub1, a recently released submergence-tolerant rice variety, has significant positive impact on rice yield when fields are submerged for 7 to 14 days with no yield penalty without flooding. Therefore, the possibility of improving submergence tolerance in Rajendra Sweta through marker assisted introgression of Sub1 locus will be beneficial for the farmers growing this variety.

**Materials and Methods**

**Layout plan**

The experiment was carried out in non-replicated trial as it was segregating material. The F2 plants along with their parents were planted in plot with spacing of 20x15 cm inter and intra row spacing. From the total 300 F2 plants 186 plants are randomly selected for the present study.

**Foreground selection for submergence tolerance**

Foreground selection was performed to identify the plants with Sub1 locus in F2 progenies of rice.

**Isolation of the genomic DNA**

Genomic DNA was isolated from the leaves of 60 days old rice seedlings using rapid DNA isolation protocol. About 100 mg of leaf samples were cut into small bits with the help of sterile scissors and transferred to sterile mortar. To this, 400 µl of 0.5 M NaOH was added and crushed. The leaf samples were finely grinded and made into paste. After crushing 1000 µl of 100 mM Tris was added. Then it was carefully transferred into a sterile 1.5 ml eppendorf tube. It was then centrifuged for 1 minute at 6000 rpm. The supernant liquid was thus obtained is used for PCR amplification.

**PCR amplification**

DNA isolated was used for PCR amplifications using Indel marker Sub1BC2 closely linked with the Sub1 gene in automated thermal cycler (Applied
Biosystems Veriti, USA). The 0.2 ml PCR tubes were arranged in the PCR tube rack and labelled for each template DNA. 1 µl of each template DNA was dispensed at the bottom of PCR tubes. The master mix for the PCR amplification was prepared in a 2.0 ml Eppendorf tube for 186 reaction plus 4 reactions extra to compensate the pipetting loss. The master mix was given a momentary spin for thorough mixing of each component. Nine µl of master mix was dispensed to each PCR tube, which is already containing 1 µl of template DNA and the final volume become 10 µl. Then 0.20 ml PCR tubes were loaded in a thermal cycler. The reaction in thermal cycler was programmed as follows:

Profile 1: 94°C for 4 minutes Initial denaturation
Profile 2: 94°C for 30 seconds Denaturation
Profile 3: 55°C for 1 minute Annealing
Profile 4: 72°C for 1 minute Extension
Profile 5: 72°C for 5 minutes Final extension
Profile 6: 12°C

Profiles 2, 3 and 4 were programmed to run for 35 cycles.

After PCR amplification, the products were resolved by agarose gel electrophoresis and banding pattern was scored.

**Agarose gel electrophoresis for separation of PCR products**

Agarose gel electrophoresis (2%) was performed to separate the amplified products. The Pyrex gel casting plate open ends were sealed with cello tape and the comb was placed properly in casting plate kept on a perfectly horizontal platform. Agarose (2%) was added to 1X TAE, boiled until the agarose was dissolved completely and then cooled to lukewarm temperature. Ethidium bromide (0.5 µg/ml) was also added as a DNA intercalating agent. It was then poured into the gel mould and allowed to solidify. The comb and the cello tape were removed carefully after solidification of the agarose.

The cast gel was placed in the electrophoresis unit with wells towards the cathode and submerged with 1X TAE to a depth of about 1cm.

**Loading the PCR products**

6µl of PCR amplified product was pipetted onto a parafilm and mixed well with 2µl of loading dye by pipetting up and down several times. Then loaded into the wells carefully with the help of a micropipette. 100bp DNA ladder also loaded as standard. The gel was run at 100 volts for 1 hour and bands were visualized and documented in gel documentation system (Uvitec gel doc system, UK). The viewed picture was photographed and saved for further analysis.

**Results and Discussion**

InDel marker Sub1BC2 was used for foreground selection of 186 F2 segregants. The results revealed that 58 plants were containing Rajendra Sweta type allele, 41 were Swarna sub 1 type allele and 87 were heterozygote type allele (Fig. 1).

Chi-square test in F2 population

Chi-square test was conducted to test the goodness of fit in the F2 population for 186 plants. It was found that the result was significant.

Indel BC1F2 marker was used to locate submergence tolerance gene contributed by Swarna Sub1 through foreground selection. In PCR, the screening of 186 F2 individuals through Indel BC1F2 marker revealed
amplification of 58 plants containing Swarna Sub1 type allele and 87 were found to be heterozygote type.

**Fig.1** Agarose gel picture of foreground selection for Sub1 locus using an InDel marker Sub1BC2 in F2 population of RajendraSweta x Swarna Sub1
The heterozygous individuals can be further advanced through selfing to get better segregants in next generations. Chi-square test was done and it was observed that segregating pattern was in the expected Mendelian segregation ratio of 1:2:1 for F_2 segregants. Similar work has been performed (Table 1). The foreground selection of F_2 segregants consists of both parental types as well as recombinant types. This reveals the Swarna Sub1 gene has been interrogated and now this population can be further used for population improvement.

**References**

Adkins, S.W, Shiraishi T, McComb JA. Submergence tolerance of rice - A new glasshouse method for the experimental submergence of plants. Physiologia Plantarum, 80: 642-646 (1990).

Singh, A., Mukul, Joshi, M., Ram, K Arya, M., Singh, P.K., Screening and evaluation of rice cultivars for submergence tolerance using ssr markers the *ecoscan*, 9(182): 255-259 (2015).

Mackill DJ, Ismail AM, Singh US, Labios RV, Paris TR. Development and rapid adoption of Submergence-Tolerant (Sub1) rice varieties. Advances Agronomy. 113:299–350. (2012)

Collard BCY, Cruz CMV, McNally KL, Virk PS, Mackill DJ. Rice Molecular Breeding Laboratories in the Genomics Era: Current Status and Future Considerations. International Journal of Plant Genomics, 10:1155-1180

---

**Table.1 Chi square test**

| Total no. of F_2 plants screened | Type of Plant/allele obtained | No. of plants | Chi square |
|---------------------------------|-------------------------------|--------------|-----------|
| 186                             | Rajendra Sweta type allele    | 58           |           |
|                                 | Heterozygotetype allele       | 87           | 3.89^16   |
|                                 | Swarna Sub 1 type allele      | 41           | (P<0.05)  |
Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics, 124:743-756 (1990).

Sinha, S., Kumar, A., Satyendra, M. K., Singh, S. P., and Singh, P. K. Screening of rice genotypes for abiotic and biotic stresses using molecular markers. *Journal of Pharmacognosy and Phytochemistry*, 7(2): 2111-2115 (2018).

Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, and Mackill DJ. Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. Annals of Botany, 103(2):151-160.

Mojulat WC, Yusop MR, Ismail MR, Juraimi AS, Harun AR, Ahmed F, Latif MA. Analysis of Simple Sequence Repeat Markers Linked to Submergence Tolerance on Newly Developed Rice Lines Derived from MR263x Swarna-Sub1. *Sains Malaysiana*, 46(4): 521-528 (2017).

How to cite this article:
Ummay Hafsa, S.P. Singh and Sweta Sinha. 2019. Foreground Selection of F2 Segregants of the Cross ‘Rajendra Sweta X Swarna Sub1’. *Int.J.Curr.Microbiol.App.Sci.* 8(11): 874-879. doi: [https://doi.org/10.20546/ijcmas.2019.811.103](https://doi.org/10.20546/ijcmas.2019.811.103)