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Foreground Selection of F_2 Segregants of the Cross 'Rajendra Sweta X Swarna Sub1'

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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 1needs 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 F_2segregants 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).

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 sevendays[1].More than 16% of rice grown in lowlands is adversely

affected by floods every year worldwide [2]. 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.[3] 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 [4].It is relatively more efficient than selection by phenotype alone[5]. 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. Introgression 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 (RajendraSweta). 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 F_2 plants along with their parents were planted in plot with spacing of 20×15 cm inter and intra row spacing. From the total 300 F_2 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 F_2progenies 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.[6]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[7]in automated thermal cycler (Applied

BiosystemsVeriti, USA).The 0.2 ml PCR tubes was 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 through mixing of the 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.

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 RajendraSweta type allele, 41 were Swarna sub 1 type allele and 87 were heterozygote type allele (Fig. 1).

After PCR amplification, the products were resolved by agarose gel electrophoresis and bandingpattern was scor

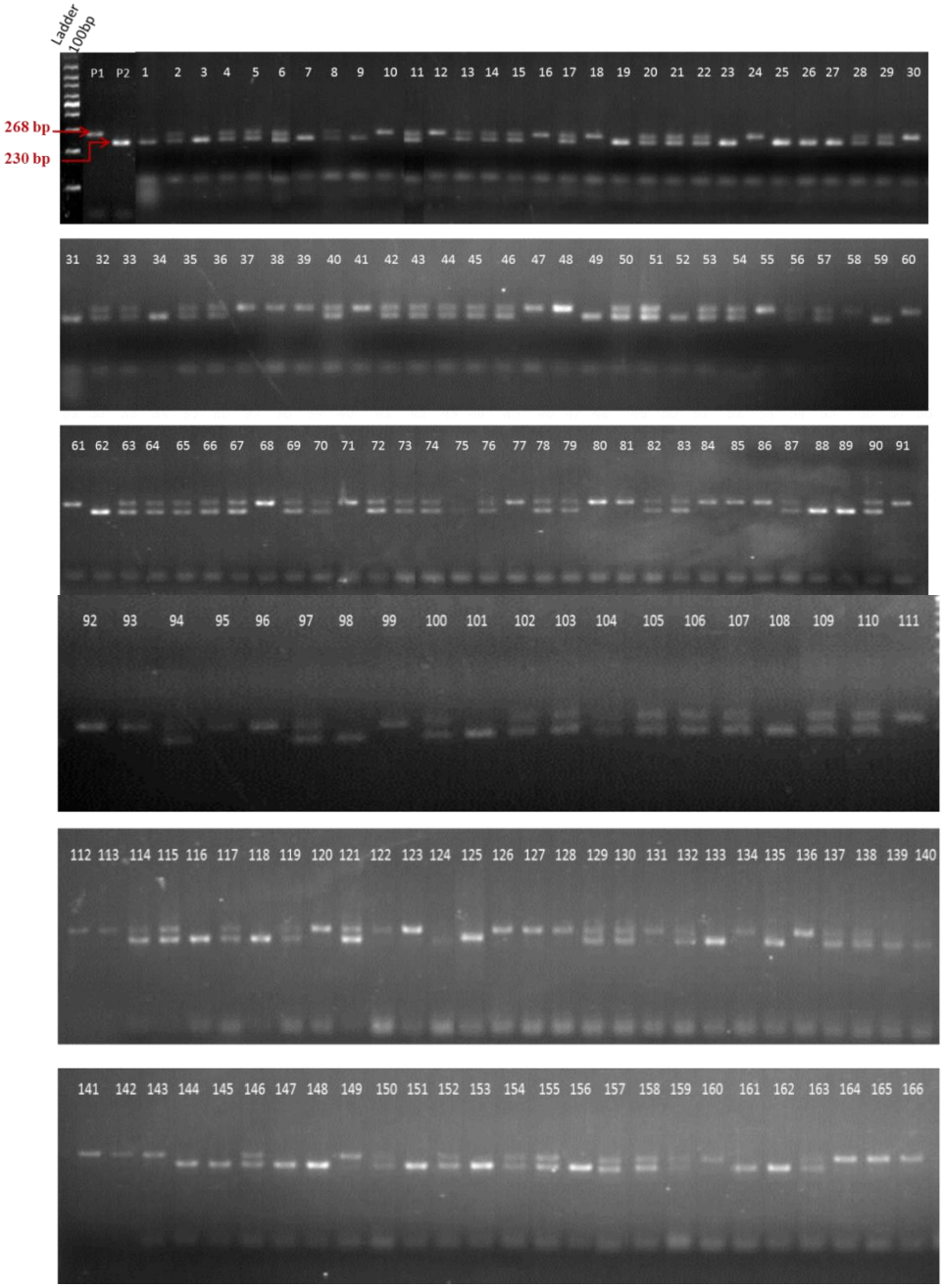
Chi-square test in F2 population

Chi-square test was conducted to test the goodness of fit in the F_2 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 F_2 individuals through Indel BC1F2 marker revealed

amplification of 58 plants containing RajendraSweta type allele, 41 individuals of 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 F_2 population of RajendraSweta x Swarna Sub1



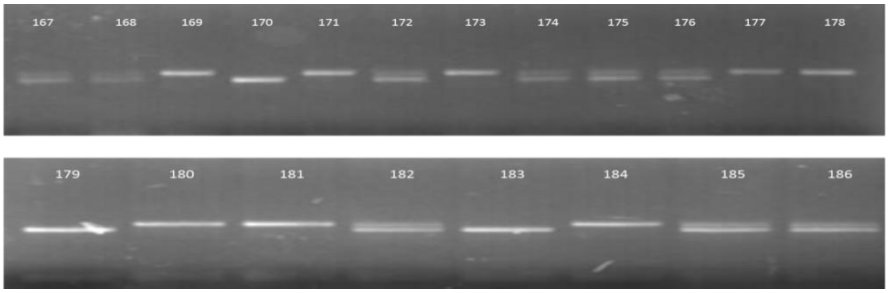


Table.1 Chi square test

Total no. of F_2 plants screened	Type of Plant/allele obtained	No. of plants	Chi square
186	Rajendra Sweta type allele	58	3.88^{NS} (P<0.05)
	Heterozygote type allele	87	
	Swarna Sub 1 type allele	41	

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 perfomed[8] (Table 1).

The foreground selection of F_2segregants 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.

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