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Short Communication

Development of Single Segment Substitution Lines (SSSLs) in rice (*Oryza sativa* L.)

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Molecular genetic linkage maps for many crop species provide the foundation and tools for gene/QTLs mapping, marker-assisted breeding, physical mapping, and map based cloning of genes etc. Introgression (or substitution) Lines (ILs) developed through molecular marker-assisted backcross breeding is an useful tool for precise mapping of QTLs in uniform genetic backgrounds [1, 4, 7]. These lines when contain only one segment from a donor, is termed as Single Segment Substitution Lines (SSSLs) or Single Segment Introgression Lines (SSILs). In this study, an attempt has been made to identify single segment substitution lines in the genetic background of 'Hua Jing Xian74' ('HJX74') a popular variety of southern China, which can be used for identification of QTLs.

The plant material consisted of BCsFa generation of crossed plant material of 8 rice varieties *viz.*, HJX74 (a *Indica-japonica* cross derivative, high yielding, semi-dwarf, medium duration variety), BG367 (long panicle, resistant to bacterial Blight), Zihui100 (contain waxy genes), IR58025B (maintainer 1ine to WA-CMS systems), Ganxiangnuo (good quality grain), IR65598-112-2 (long grained), Star bonnet99 (better plant type) and Brazil upland (tolerant to weed infestation). Of these, HJX74 was used as recurrent parent and other seven as donor parent to produce SSSLs in the genetic background of HXJ74. The plant population used was divided into seven groups *viz.* W4 (BG367), W5 (Zihui100), W12 (IR58025B), W17 (Ganxiangnuo), W21 (IR65598-112-2), W24 (Star bonnet99) and W32 (Brazil upland rice). Each group had 2, 8, 1, 11, 14, 8 and 8 lines, respectively and 20 plants were grown for each line. 168 Simple Sequence Repeats (SSR) markers with designation of Rice Microsatellite (RM) and five Oryza Simple sequence' Repeats (OSR) were used to detect substitution segments. Rice Microsatellite map developed at Cornell University, USA (known as Cornell map) was used as benchmark map [6]. The PSM markers were designed in Plant Molecular Breeding Research Center, South China Agricultural University (SCAU), Guangzhou, China [2]. The crop was raised in the February to June season of the year 2003 at experimental farm, SCAU, Guangzhou, China.

For extraction of DNA, mini-scale method [8] was used with minor modification. The leaf samples were collected from about 30 days old seedlings. The leaf samples were grinded with a mortar adding 1200ul of extraction buffer (50mM TRIS-HC1 of pH 8.0, 25mM EDTA, 300mM NaCl, and 1% SDS). The grinded fine mass was transferred to a centrifuge tube, added one volume of Chloroform, Isomyl alcohol (24:1) to deproteinize the lysate, and centrifuged to separate the debris. The supernatant were collected and DNA was precipitated with cold absolute ethanol. The DNA pellets were washed with 75% ethanol, air-dried and finally re-suspended in 100-300 µl of TE buffer (10mM TPJS-HC1 pH 8.0, 1mM EDTA pH 8.0) to attain a concentration of 50mg/µl and stored in –20°C for use. The PCR was carried out with 20µl reaction volume in PTC 100 Programmable Thermal Controller (MJ Research Inc.). The 20ul PCR reaction volumes contained 2.0µl of template DNA (50 ng), l.5µl of each primer (50ng), 0.4µl dNTP (0.05mM), 2µl of 1 × PCR buffer (10mM TRIS, pH 8.0; 50mM KCl; 1.8mM $MgCl_2$ and 0.01 mg/ml gelatin), and one unit of *Taq* DNA polymerase. The PCR was conducted as per [5] with little modification. The PCR products (amplicon) were mixed with 10 × loading dye (bromphenol blue) and resolved through electrophoresis on 6% denaturing polyacrylamide gel. The electrophoresis was conducted for about 3 hours (250 V) with 1 × TBE buffer and gels were subjected to silver staining in 0.1% $AgNO_3$ solution and slowly stirred for 10 minutes. The gels were then transferred to developing solution (1.5 NaOH, 0.019g Disodium tetraborate decahydrate, 0.4ml Formaldehyde per 100 ml water) to display the bands. Identification of substitution segments was made in

BC_5F_2 generation. A population of 1040 plants was surveyed by using 174 SSR markers. Out of the 174 markers, 19 markers could not produce any bands hence; only 155 markers were used to locate the substitution segments. Electrophoresis bands were scored as 1 = homozygous plant with recurrent parent genome, 2 = plant with heterozygous substituted segment and 3 = plant with substituted segment of donor parent. Out of 1040 plants, substituted segments were detected in 854 plants. The highest numbers of plants (241) with substituted segments were recorded in W21 group followed by 172 in W17, 147 in W32, 125 in W5, 120 in W24, 35 in W4 and 14 in W12. These plants with substituted segment were further classified into plants having single segment (69.58%), double segment (42.00%) and multiple segment (10.71%). Data was also analyzed for number of substituted segments per plant. A total of 1342 substituted segments were found in the whole population. The highest number of average substitution segments per plant was 2.14 in W32 group followed by 1.58 in W21, 1.51 in W17, 1.48 in W4, 1.35 in W5 and 1.00 in W12. The total number of substitution markers was 1764, which were distributed over all the seven groups as 548 in W21, 415 in W32, 299 in W17, 246 in W5, 58 in W4 and 14 in W12. The average number of substitution markers/segment was 1.45, 1.43, 1.31, 1.22, 1.15, 1.11 and 1.00 in W5, W21, W32, W24, W17, W14 and W12 groups, respectively.

The distribution of substitution segments over all the 12 chromosomes showed that segments were randomly distributed across all the chromosomes. The highest number of substituted segments was found in chromosome 6 followed by chromosome 12, 3, 8, 9, 10, 2, 5, 4, 11, 1 and 7. The average number of substituted segments per chromosome was recorded 31.33 in W21 followed by 26.33 in W32, 21.66 in W17, 14.08 in W5, 12.50 in W24, 4.33 in W4 and 1.16 in W12. This showed that chromosomes like 1, 2, 4, 7 and 11 are poorly represented in the single segment survey and therefore, in spite of having maximum length have very less substituted segments. More populations need to be developed and surveyed for these chromosomes in the subsequent generations. Characterization of the substitution segment is necessary to trace the recovery of the recipient parent genome with the substituted donor segments. However, since most of the segments were heterozygous and will segregate in subsequent generation, estimation of segment length as well as donor genome contribution and recovery of recurrent parent genome should be done only in homozygous single segment substitution plants. The substitution lines carrying single segment not only allow plant breeder to estimate the action and interaction of QTLs in the uniform genetic background but it offer an added advantage to study the traits having epistatic interaction which is otherwise very difficult to detect and estimate.

In this study, 106 plants with homozygous single segment of donor parent were marked and separated. The number of plants varied in each genotype group and it was two in BG367, 14 in Zihui100, four in IR58025B, 23 in Ganxiangnuo, 33 in IR65598-112-2, 22 in Star bonnet 99 and eight in Brazil upland rice. These plants can be directly developed into populations to identify QTLs in the next generation. However, to ensure that the SSSLs contain one and only segment of donor chromosome, the population need to be tested either by residual segment survey by using 4-5 random markers per chromosome or by phenotype survey for the co-segregation of the lines with markers. Lines having more than one segment after residual segment survey and not co-segregating with the markers will be removed from the final population. The approach has also greater use when substitution lines identified with desired gene are present in superior agronomic genetic background because such lines with minimum testing can be put into varietal trials and released as commercial cultivars in short time.

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