Identification of genetically diverse genotypes for photoperiod insensitivity in soybean using RAPD Markers

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ABSTRACT

Most of the Indian soybean varieties were found to be highly sensitive to photoperiod, which limits their cultivation in only localized area. Identification of genetically diverse source of photoperiod insensitive would help to broaden the genetic base for this trait. Present study was undertaken with RAPD markers for genetic diversity estimation in 44 accessions of soybean differing in response to photoperiod sensitivity. The selected twenty-five RAPD primers produced a total of 199 amplicons, which generated 89.9 % polymorphism. The number of amplification products ranged from 2 to 13 for different primers. The polymorphism information content ranged from 0.0 for monomorphic loci to 0.5 with an average of 0.289. Genetic diversity between pairs of genotypes was 37.7% with a range of 3.9 to 71.6%. UPGMA cluster analysis placed all the accessions of soybean into four major clusters. No discernable geographical patterns were observed in clustering however; the smaller groups corresponded well with pedigree. Mantel’s test (r = 0.915) indicates very good fit for clustering pattern. Two genotypes, MACS 330 and 111/2/1939 made a very divergent group from other accessions of soybean and highly photoperiod insensitive that may be potential source for broadening the genetic base of soybean for this trait. [Physiol. Mol. Biol. Plants 2008; 14(4) : 369-375] E-mail : ikrps@yahoo.com

Key words : Genetic diversity, Photoperiod, RAPD, Soybean, UPGMA

INTRODUCTION

Soybean is the first largest oil seed crop in India and played a major role in up-liftment of socio-economic status of Indian farmers. Indian soybean has to be globally competitive in present era of opening up of world trade and liberalized economy. India produced 5.60 mt of soybean in an area of 6.0 mha during 2001-2002 with an average of 0.93 t/ha and contributed 3.17 % of total global production (Singh et al., 2006). Soybean producing regions in India range from the lower Himalayan Hills and Northern Plain in the north to the Deccan Plateau in south. The soybean varieties cultivated in these areas were developed through separate breeding programmes, because most of the Indian soybean varieties (> 95%) were found to be highly sensitive to photoperiod that limits their cultivation in only localized area (Bhatia et al., 2003). The photoperiod response is a major criterion, which determines the latitudinal adaptation of a soybean variety (Hartwig 1970; Hartwig and Kiihl, 1979).

Identification of diverse genotypes is the prerequisite for improvement of any trait in the crop plants. Further more, monitoring the genetic variability within gene pool of elite breeding material could make crop improvement more efficient by the direct accumulation of favored alleles. DNA markers are being increasingly utilized in cultivar development, quality control of seed production, measurement of genetic diversity for conservation and management, varietal identification and intellectual property protection (IPP). Recent studies have used molecular markers to help in identification of genetically diverse genotypes to use in crosses in cultivar
improvement programme. These studies have more success than conventional selection programme in producing productive lines from plant introduction/exotic lines crosses with elite lines. There are several reports of using molecular markers for evaluation of soybean germplasm. RAPD markers have been shown to be a simple and effective means to evaluate variability in crops. RAPD are well suited for diversity studies because they are technically simple, non radioactive, relatively inexpensive, and require small quantity of DNA (Chen and Nelson, 2005). Previously, RAPD markers were used for evaluation of genetic diversity in soybean (Thompson and Nelson, 1998; Thompson et al., 1998; Brown-Guidera et al., 2000; Li et al., 2001; Li and Nelson, 2001, 2002; Chen and Nelson, 2005; Singh et al., 2006). In the present study, a concerted effort were made firstly to study the genetic relationships among the screened photoperiod insensitive and sensitive accessions of soybean germplasm belonging to different countries and secondly, identification of genetically diverse source of photoperiod-insensitive genotypes using RAPD markers for their further utilization in breeding programme for development of soybean cultivars.

MATERIALS AND METHODS

Plant Materials

One thousand soybean genotypes assembled from India, USA, Hungary, Philippines and Taiwan were screened for sensitivity to photoperiodism measured as days to flowering in ambient as well as in extended photoperiod (17 hrs) created artificially at the Experimental Farm of National Research Centre for Soybean, Indore (22.40° N), India for two years (2002-03 and 2003-04). Each genotype was sown in single row plots of 1 m long with 0.6 m row to row and 5 cm plant-to-plant distance. Recommended package of practices for the region were followed. The average photoperiod from planting to mean days to flowering under ambient conditions was 13.2 hrs. Extended photoperiod of 17 hrs was created by providing lighting with 40-watt incandescent bulbs at a height of 3 feet above the crop canopy and the bulbs were connected to an automatic timer. Data on the appearance of all the phenological stages (Fehr et al., 1971) was recorded for all the genotypes grown under ambient and extended photoperiods. The degree of sensitivity to photoperiod among the genotypes was determined on the basis of number of day’s delay in flowering under extended photoperiod as compared to ambient photoperiod (Bhatia et al., 2003). Out of the 1000 genotypes, 15 were found to show different degree of photoperiod insensitivity and remaining 985 were sensitive. In the present experiment 44 genotypes, comprising 15 genotypes showing different degree of photoperiod insensitivity and 29 sensitive genotypes were selected for analysis using RAPD markers. The place of origin and number of days delay in flowering from ambient to extended photoperiod is given in Table 1. Ten leaves, one each from ten plants of 44 soybean genotypes were collected and DNA was isolated by the method described by Doyle and Doyle (1990).

DNA amplification

Arbitrary decamer primers from Operon Technologies Inc., Alamenda, CA, USA were dissolved in sterilized T10E1 (10mM Tris-Cl, 1mM EDTA, pH 8.0) to a concentration of 15 ng/μl. Twenty five primers from Operon kits (13 from AA, 7 from B, 1 from E and 4 from F series) were used for RAPD amplification as described by Williams et al. (1990). Amplification was carried out in a 25μl reaction volume containing 1X PCR assay buffer (50 mM KCl, 10 mM Tris-Cl, 1.5 mM MgCl2), 200 μM of each dNTPs, 0.6 μM primer, 0.5 units Taq polymerase (Bangalore Genei Pvt. Ltd Bangalore, India) and 30 ng of DNA template (conc. approximately 15 ng/μl). The amplification reaction was carried out in a peltier controlled thermal cycler (M J Research, Model PTC-200). The first cycle consisted of denaturation of template DNA at 94°C for 4 min. followed by primer annealing at 37°C for 1 min. and primer extension at 72°C for 2 min. In the next 43 cycles, the period of denaturation was reduced to 1 min while the primer annealing and the primer extension time remained as in the first cycle. The last step was primer extension at 72°C for 8 min. PCR products were separated on a 1.5 % agarose gel containing ethidium bromide using 1X TAE buffer. The sizes of the amplified fragments were determined by using DNA size standards (1 kb DNA ladder, MBI Fermentas, Lithuania). DNA fragments were visualized and photographed using gel documentation system (Gene Genius, Bio Imaging System, Synaptics Group, UK). To test the reproducibility of the profiles, the reactions were repeated at least twice.

Data Scoring and Statistical analysis

The RAPD products were scored as present (1) or absent (0) for each primer – genotype combination. The data entry was done into a binary data matrix as discrete variables. The Jaccard’s similarity coefficient values (Jaccard, 1908) for each pairwise comparison between