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VOL. 7, NO. 11, NOVEMBER 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

ESTIMATING OF RAPD MARKER ASSOCIATED TO COLOR GENE IN *Zinnia Elegans* JACQ

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ABSTRACT

Flower color contributes mainly to the market value of ornamental plants including *Zinnia elegans* Jacq. The coloration of flowers is determined by several genes. The previous study showed that Zinnia coloration is controlled by two independent genes, which are white color as dominant (chromogen) and its suppressor. The aim of this study is to investigate marker that associated with chromogen based on RAPD. DNA from each of 240 individuals in M2 population was extracted from leaf by CTAB method (Doyle and Doyle, 1990). Fifteen primers were used to amplify DNA fragments. The PCR program was consisted of preheating at 94˚C for 1 min, followed by 45 cycles of denaturation at 94˚C for 30 sec, annealing at 38˚C for 30 sec, and elongation at 72˚C for 1 min 30 sec. The last cycle was followed by a final extension cycle at 72˚C for 7 min. Six among fifteen primers were selected and produced 60 loci which is 100% was polymorphic. The binary data were analyzed using Fisher Exact Test and Kendall Tau correlation in the SAS program to get correlation between markers and those traits. The result showed $OPB17_{1400}$ and $OPA18_{1500}$ markers were associated with chromogen in Zinnia.

Keywords: *Zinnia elegans* Jacq, flower color gene, association, primer, RAPD.

INTRODUCTION

Flower color is one of the most important traits in ornamental plants, dictating consumer interest and attraction. As such, it is a critical factor for the commercial success of plants on the market. The inheritance of color in flowers is a large field, especially, as it varies in different species. Color can be dominant, recessive, or additive in nature, in different species, and in some plant species it can be a combination of these factors. Also some plants have two or more different genetic defects to make them white or non-colored.

In general, a DNA marker linked to specific gene was developed by using near-isogenic lines (NILs), and their genetic linkage map at the specific gene (Young *et al*., 1988; Martin *et al*., 1991; Paran *et al*., 1991). Various markers have been used to associate with different traits of crop plants. Michelmore *et al*. (1991) used bulk segregant analysis technique to identify or link markers with various traits in many plants. DNA markers have been linked to fruit skin color in pear (Inoue *et al*., 2006), apple (XiaoWei *et al*., 2009) and grapes (Ren *et al*., 2000). Markers have been found to be associated with resistance for scab (Sestras *et al*., 2009) and columnar growth habit in apple (Costa *et al*., 2001). RAPD markers linked to sex were identified in pistachio (Kafkas *et al*., 2001) and guggal (Samantaray *et al*., 2010). QTLs were identified for grain yield in rice (Venuprasad *et al*., 2009), pod and kernel traits in peanut (Selvaraj *et al*., 2009) and rust resistance in eucalyptus (Zamprogno *et al*., 2008). Markers for dwarfing in pear (YanLi *et al*., 2007), early flowering in eucalyptus (Domingues *et al*., 2006), pollen sterility in peach (Jun *et al*., 2004), precocity in walnuts (KeQiang *et al*., 2002) and seedlessness in grape (Mejia and Hinrichsen, 2003) were also identified and reported.

Kendall's tau value is defined as (concordant - discordant)/ (concordant + discordant) values (Kendall, 1970). This value will be high (close to one) in the case of a positive relationship, positive meaning that both values are increasing in the same direction. Because it only involves the relative orderings of similarity values, it is relatively insensitive to `outliers'. The aim of this study is to investigate marker that associated with chromogen based on RAPD using Fisher Exact Test and Kendall Tau correlation.

MATERIALS AND METHODS

Plant materials and DNA extraction

Total DNA was isolated by a modified CTAB method (Doyle and Doyle, 1987). DNA taken from each of 240 individuals in M2 population was extracted from leaf. The primers used for PCR amplification are OPA 18, OPB 01, OPB 07, OPB 10, OPB 17 and OPB 20. The PCR included preheating at 94˚C for 1 min, followed by 45 cycles of denaturation 94˚C for 30 sec, annealing at 38˚C for 30 sec, and elongation at 72˚C for 1 min 30 sec. The last cycle was followed by a final extension cycle at 72˚C for 7 min. Random 10-base primers (Operon technology Inc.) were independently used for the polymerase chain reaction of RAPD analysis. Polymerase chain reactions were conducted in a volume of 10 µl containing 5 µl PCR *mix* Go Taq® Green (*Promega*), 0.25 µl 100 µM primer (*Sigma-Proligo*), 2.25 µl DNA genome as a template and 2.5 µl nuclease free water. The amplification products were analyzed by electrophoresis in 1.5% agarose gels (NuSieve 3:1 agarose). The gels were stained with ethidium bromide, visualized under ultraviolet light and documented by camera. The RAPD bands were scored 1 as present or 0 as absent. The amplified fragments were

evaluated using Fisher Exact Test and Kendall Tau correlation in SAS program to get correlation between markers and coloration phenotypes.

RESULT AND DISCUSSIONS

Two color phenotypes (colored phenotypic and colorless phenotypic) were observed in populations M_2. There were 205 plants with colored flower and 35 plants with colorless (white). (Figure-1).

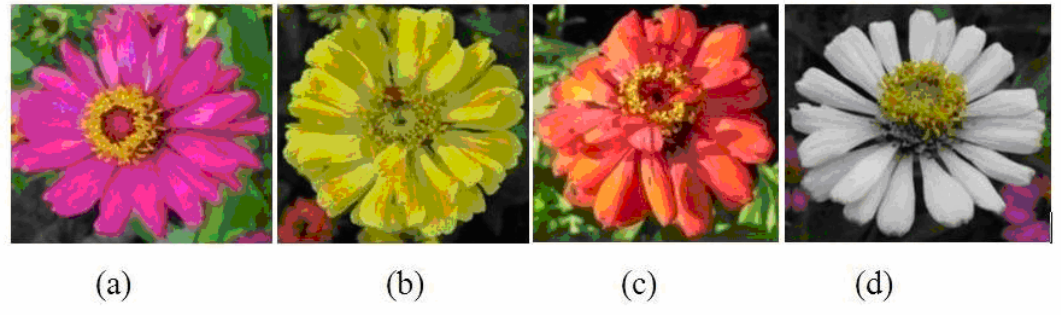

Figure-1. Two kinds of flower phenotype in Zinnia: coloured type (a,b,c); colourless type (d).

To identify specific RAPD markers associated with flower color phenotypes, 15 random primers were screened. Six primers showed polymorphism bands. Those primers could amplified 60 bands which had molecular weight between 200 and 1900 bp (data not shown) (Figure-2).

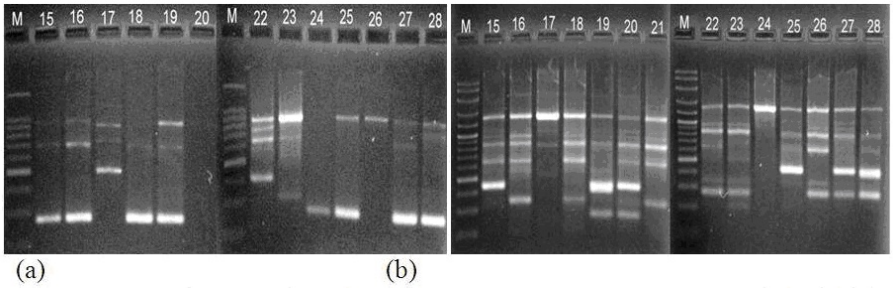

Figure-2. Agarose gel electrophoresis of amplification products generated by primer OPB 07 (a) and OPB 17 (b).

The markers present in one of the bulks and absent in the corresponding bulk of contrasting phenotype were considered as polymorphic and identified as a putative marker for that flower coloration. Based on the Fisher Exact test and Kendall tau correlation, the primer OPA18, OPB17 and OPB 07 have association with the coloration of Zinnia flower. OPA18, and OPB17 were associated with colorless (white) and OPB07 was associated with colored. (Table-1).

Table-1. A RAPD primer has associated with coloration flower in M2 population (Fisher Exact Test 5% dan Kendall Tau correlation).

No.	Primers	Fisher exact test	Kendall Tau correlation
1	OPA18-1500	0.0120	-0.1917
2	OPB17- 1400	0.0000	-0.3716
3	OPB07-200	0.0233	0.1560

This result supports a model involving two major genes proposed by Boyle and Stimart (1988). Presence of the anthocianidins pelargonidin and cyanidin is controlled by single dominant gene (An1). Carotenoid expression is conditioned by a recessive gene (ca) governing its presence and other genes controlling the distribution of carotenoid in ligules.

RAPD marker linked with colored phenotype (OPB 07_{-200}) and two RAPD markers ($OPB17_{-1400}$ and $OPB18_{-1500}$) assosiated with colorless phenotype were obtained from M_2 population. Following the hypothesis of Boyle and Stimart (1988) this marker should be linked to a major gene on C locus (called modifier) that has the dominant effect on suppressing the colored flower. Those

phenotypes were exhibited when the modifier gene worked incompletely.

We found that two RAPD markers (OPB18-$_{1500}$ and OPB17-$_{1400}$) could be used to select colorless with probability as high as approximately 19% and 37%, respectively. The marker was apparently useful for the putative markers individuals in a breeding program study of coloration in Zinnia flower. This is a first report on developing a DNA marker associated to the flower coloration phenotype in Zinnia. This experiment suggests the possibility of utilizing the DNA marker even in plant species, which have a highly heterozygous genome, without requiring a genetic linkage map and any DNA sequence information. Therefore, it seems to be an effective technique for conveniently developing selection markers in coloration of flower in Zinnia. In conclusion, we are now found that OPB18-$_{1500}$ and OPB17-$_{1400}$ associated with colorless.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the financial support from the Department of Breeding Program Gadjah Mada University, for this investigation. Thanks to Mustaqim as a technical Assistance.

REFERENCES

Boyle T.H. and St imart D. P. 1988. Inheritance of Ray floret in Zinnia Angustifolia HBK and Z. elegans Jacq. Journal of Heredity. 79: 289-293.

Costa F., Pancaldi M. and Sansavini S. 2001. Identification of RAPD marker associated with columnar growth habit of apple. Rivista-di-Frutticoltura-e-di-Ortofloricoltura. 63(5): 95-97.

Domingues D.S., Farro A.P.C., Coscrato V.E., Mello E.J., de Oda S. and Marino C.L. 2006. Identification of RAPD and SCAR marker related to early flowering in Eucalyptus grandis. Ciencia Florestal. 16(3/4): 251-260.

Doyle J.J. and J.L. Doyle. 1990. Isolation of plant from tissue. Focus. 12: 13-15.

Inoue E., Kasumi M., Sakuma F., Anzai H., Amano K., Hara H. 2006. Identification of RAPD marker linked to fruit skin color in Japanese pear (Pyrus pyrifolia Nakai). Sci. Hortic. 107(3): 254-258.

Jun J.H., Chung K.H., Jeong S.B. and Lee H.J. 2004. An RAPD marker linked to the pollen sterility gene ps in peach (Prunus persica). J. Hortic. Sci. Biotechnol. 79(4): 587-590.

Kafkas S., Cetiner S. and Perl-Treves R. 2001. Development of sex associated RAPD markers in wild Pistacia species. J. Hortic. Sci. Biotechnol. 76: 242-246.

Kendall M. G. 1970. Rank Correlation Methods, 3rd edn. London: Charles Griffin.

KeQiang Y., YueJin W., YinDong Z. and XueQin Z. 2002. RAPD analysis for the identification of the precocious trait in walnuts. Acta Hortic. Sin. 29(6): 573-574.

Martin G.B., Williams J.G.K. and Tanksley S.D. 1991. Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Genetics. 88: 2336-2340.

Mejia N. and Hinrichsen P. 2003. A new highly assertive SCAR marker potentially useful to assist selection for seedlessness in table grape breeding. Acta Hortic. 603: 559-564.

Michelmore R.W., Paran I. and Kesseli R.V. 1991. Identification of markers linked to disease resistant genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions using segregating populations. Proc. Natl.Acad. Sci. U. S. A. 88: 9828-9832.

Paran I., Kesseli R. and Michelmore R. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce using near-isogenic lines. Genome. 34: 1021-1027.

Ren Z., Lamikanra O. and Lu J. 2000. Identification of a RAPD marker closely linked to the fruit color in muscadine grapes (*Vitis rotundifolia*). Acta Hortic. 528: 263-266.

Samantaray S., Geetha K.A., Hidayath K.P. and Satyabrata Maiti. 2010. Identification of RAPD markers linked to sex determination in guggal [Commiphora wightii (Arnott.)] Bandar. Plant Biotechnol. Rep. 4: 95-99.

Selvaraj M.G., Narayana M., Schubert A.M., Ayers J.L., Baring M.R. and Burow M.D. 2009. Identification of QTLs for pod and kernel traits in cultivated peanut by bulked segregant analysis. Elect. J. Biotechnol. 12: 2.

Sestras R., Pamfil D., Ardelean M., Botez C., Sestras A., Mitre I., Dan C. and Mihalte L. 2009. Use of phenotypic and MAS selection based on bulk segregant analysis to reveal the genetic variability induced by artificial hybridization in apple. Not. Bot. Horti Agrobotanici, Cluj-Napoca. 37(1): 273-277.

Venuprasad R., Dalid C.O., Valle M., Zhao d., Espiritu D., Sta Cruz M., Amante M.T., Kumar M. and Atlin A.G.N. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. Theo. Appl. Genet. 129(1): 177-190.

XiaoWei H., CaiHong W., YiKe T. and Hui W. 2009. A SSR marker related to the traits of skin color in apple. J. Fruit Sci. 26 (3): 379-381.

YanLi J., CaiHong W., YiKe T., Dai HongYi D. and Liang W. 2007. A SCAR marker linked to pcDw gene in pear. Acta Hortic. Sin. 34(6): 1531-1534.

Young N.D., Zamir D., Ganal M.W. and Tanksley S.D. 1988. Use of isogeniclines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. Genetics. 20: 579-585.

Zamprogno K.C., Furtado E.L., Marino C.L., Bonine C.A. and Dias D.C. 2008. Use of bulked segregant analysis in identification of molecular markers linked to resistance to rust (Puccinia psidii winter) in Eucalyptus sp. Summa Phytopathol. 34(3): 253-255.