June 2009 Volume 3 Issue 2

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Minireview

BCAIJ, 3(2), 2009 [48-52]

RAPD analysis of phylogenetic relationship(s) and genetic variation(s) in the genus *Allium*

Ganesh G.Adsul*, Akhilesh K.Chaurasia, Anil V.Dhake, Ramanlal M.Kothari
Jain Hi-tech Agri Institute, Agri Park, Jain Hills, Jain Irrigation Systems Ltd, Jalgaon 425001 (INDIA)
Tel : +91-257-2260288 ; Fax: +91-257-2264920
E-mail : biotech@jains.com
Received: 29th April, 2009 ; Accepted: 4th May, 2009

ABSTRACT

This mini-review has highlighted the importance of onions as vegetable and has shown its taxonomic position among 750 species of genus *Allium*. It has compared merits of Random Amplified Polymorphic DNA (RAPD) markers vs Restriction Fragment Length Polymorphism (RFLP) and established the superiority of RAPD over RFLP. Further, significance of RAPD markers in onion breeding program is indicated and subsequently validated by its multi-faceted applications to (i) assess in-bred integrity, (ii) establish genetic relationship in conjunction with morphological markers, (iii) prove hybrid status in conjunction with genomic *in-situ* hybridization (GISH), (iv) established origin and relationship between two taxa, (v) establish phylogenetic relationship, (vi) confirm inter-specific hybrids, (vii) assess genetic diversity among the onion cultivars and (viii) prepare genetic map of onion cultivars.

KEYWORDS

RAPD;
RFLP;
GISH;
Morphological characters;
Status of onion sp.

INTRODUCTION

Onions are one of the oldest vegetables known to mankind, used for their flavor, aroma, texture and taste. They are used in (i) a vast number of recipes and preparations, spanning world's almost all cultures, (ii) fresh, frozen, fried, roasted, dehydrated, canned and pickled states and (iii) usually granular, chopped or sliced forms, in almost every type of food, including fresh salads, cooked foods, as a spicy decoration and an accompaniment to the main course[1]. Depending on the variety, an onion can be spicy and pungent or mild and sweet, preserved domestically or industrially as a raw material for a variety of food manufacturing processes. In fact, onions were one of the earliest (since 1941) established dehydrated vegetables and widely used in the manufacture of other processed foods[2].

Onions are cultivated species, though their wild related species are still found in the areas, which are regarded as botanical centers of origin for the crop: the south western part of central Asia, largely covered by countries of Iran, Afghanistan, Pakistan and Southern Republics of the former Soviet Union[3].

Taxonomic position of onion

Genus *Allium* contains more than 750 species, rendering it a taxonomically complicated genus. Taxonomic position of onion after molecular analysis is summarized in TABLE 1[4].

TABLE 1: Taxonomic position of onion

1	Class	Liliopsida
2	Sub-class	Liliidae
3	Super order	Liliianae
4	Order	Amaryllidales
5	Family	Alliaceae
6	Sub-family	Allioideae
7	Tribe	Allieae
8	Genus	*Allium*
9	Species	*cepa*

RAPD (Radom amplified polymorphic DNA) markers

Williams et al.[5] and Welsh and McClelland[6] were the first to independently develop polymorphic markers-RAPDs. In RAPD, decamer random primers were utilized in PCR to generate polymorphism. Primer must bind at two sites in opposite orientation so as to produce each RAPD band. Absence of a particular band results from differences in DNA sequences between individuals in primer-binding site[7]. These are easily and economically assayed and segregate in Mendelian fashion. Thus, RAPD markers behave as dominant markers and are more useful than other methods, mainly because they:

1. Require little time.
2. Involve utilization of universal set of primers, facilitating easy screening.
3. Require small amount of DNA[8].
4. Require less investment in laboratory equipment and less labor
5. Use a safer technique, free from danger(s) of radioactivity[9].

Why RAPD markers are useful in onion breeding program?

As a result of paucity of available genetic markers, onion breeding still relies heavily on phenotypic selection methods. However, in recent years, polymorphic DNA markers have made a major contribution to plant improvement programs, particularly markers, based on Restriction Fragment Length Polymorphisms (RFLPs). The RFLP-based linkage mapping in *Allium* species, however, presents certain problems associated with the relatively large size of nuclear genome relative to most other herbaceous crops. The un-replicated level of DNA per cell (2C content) in *A.cepa* is 33.5 pg compared with merely 2.0 pg in tomato (*Lycopersicon esculentum*) or 7.8 pg in maize (*Zea mays*)[10]. This means that sensitivity of the RFLP assay to reveal low-copy-number sequences in onion must be several times greater than that for other crops. Moreover, generation of single-copy sequence probes from onion is difficult, because proportion of the genome comprising of such sequences is very small. Although researchers on other species were successful at targeting low-copy-number sequences to prepare genomic libraries[11], attempts to do the same in onion were largely unsuccessful. In contrast, random amplified polymorphic DNA (RAPD) markers[7] were useful for genetic studies in *Allium* due to several merits[12].

Merits of using RAPD markers

RAPD (Randomly Amplified Polymorphic DNA) has been utilized (a) for the analysis of different species without requirement of prior knowledge of genome to be investigated and (b) by virtue of quick and comparatively economical approach to detect even small genetic difference(s)[13,14]. These inherent merits enabled onion scientists to utilize RAPD for cultivar or line identification in different onion breeding programs (*Allium cepa*[15,16]; *Allium sativum*[17,18]; triploid onion[19]). It is, therefore, interesting to know as to how RAPD procedure was used.

Isolation of DNA and RAPD procedure

RAPD analysis was applied to onion *(Allium cepa)* and other *Allium* species in order to (i) assess the degree of polymorphism within the genus and (ii) investigate if this approach was suitable for genetic studies of onion. For this purpose, seven cultivars of *Allium cepa* were chosen, including shallot and single cultivar of Japanese bunching onion (*Allium fistulosum*), chive (*Allium schoenoprasum*), leek (*Allium ampeloprasum*) and onion wild species (*Allium roylei*). The extraction of onion DNA of reasonable purity and quality being difficult, several methods were attempted, including extraction from isolated nuclei[20], which required fresh starting material and gave low yield of DNA. With the exception of isolation of nuclei, other methods gave rise to an extract highly contaminated with polysaccharides and other impurities. Modification of the method of Saghai-Maroof et al.[21] by including caesium chloride density gradient centrifugation for purification[22], gave DNA of reasonable purity and yield. RAPD was done using Techne (UK) PHC-2 programmed thermocycler

Minireview

(1 cycle of 3 min denaturation at 94^{0}C, 45 cycles of 1 min at 94^{0}C, 1 min annealing at 33^{0}C, 2 min extension at 73^{0}C and final extension at 73^{0}C for 5 min, followed by slow cooling to ambient temperature). Seven out of twenty primers revealed scorable polymorphisms between cultivars of *A.cepa*, resulting in a broad agreement with previous classifications of the species studied, confirming the validity of the method[12].

Use of RAPD to assess in-bred integrity

Commercial onion bulb growers had complained that hybrids grown successfully by them for few years failed to give expected yields. Therefore, RAPD was used for assessing in-bred integrity. The assessment showed that in-breds used to produce hybrid-onion seeds, (i) rarely self-pollinated for more than two generations, (ii) entertained high level of heterozygosity and (iii) selection, drift or contamination over the period affected their performance. Thus, RAPD markers identified between two in-bred onion lines, were used to examine changes in independently maintained and publically released in-bred onion lines and their Mendelian inheritance demonstrated, which revealed contamination, contributing to lower yields[23].

RAPD in conjunction with morphological markers for assessing genetic relationship

The *Allium cepa* species included two major crops on the basis of their morphological traits and typical reproduction mode: (i) sexually reproduced biennial onions and (ii) vegetatively propagated perennial shallots, which rarely flower. In addition, the seed-propagated shallot, a recently released variety, with an intermediate phenotype for life history, has been used by breeders. European and tropical accessions, besides these species were analyzed using molecular markers (RAPD) and morphological characters of growth and development. Morphological data recorded from onions and vegetatively propagated shallots was submitted to multivariate statistical analysis. Results indicated that seed-propagated shallot was more closely related to onions than to vegetatively propagated shallots, besides a geographical genetic diversity[24].

Use of GISH and RAPD to prove hybrid status

Three vegetative crops of *Cepa* in genus *Allium* (top onion, French grey shallot and viviparous triploid onion) of suspected hybridogenic origin were studied, using genomic *in situ* hybridization (GISH) and RAPD markers. The results showed that (i) in *A.x proliferum*, parental chromosomes were derived from *A.fistulosum* and *A.cepa* as unequivocally identified by GISH to prove hybrid status of this crop and (ii) French grey shallot belonged to *A.oschaninii* on the basis of RAPD analysis[13].

Use of RFLP and RAPD to establish origin and relationship between two taxa

The origin of *Allium fistulosum* (bunching onion) and its relation to *Allium altaicum* was examined by (a) RFLP analysis of five non-coding cpDNA regions and (b) RAPD analysis of nuclear DNA. While RFLP analysis could distinguish the two species, only RAPD analysis clarified the inter-relationship between the two taxa[25].

Use of RAPD and PCR-RFLP analysis to establish phylogenetic relationship

RAPD and PCR-RFLP analysis was also used to establish phylogenetic relationship among collected accessions of shallot and *Allium x wakegi*, as also to assess its origin. The results indicated that (i) out of 100 primers, only 20 amplified with 112 scorable bands for cluster analysis, (ii) out of 2 main cluster groups, only one group belonged to shallots, while another to *A.xwakegi* and (iii) sub-groups of clusters reflected phenotypic differentiation in shallots and regional specificity in some *A. x wakegi* accessions[26].

Use of RAPD to confirm inter-specific hybrids of onion

Inter-specific hybridization, performed between wild and cultivated species of genus *Allium* generated hybrids, possessing characteristics of both parental plants, as judged by RAPD analysis[27] Similarly, inter-specific hybrids (2*n* = 16) between *Allium fistulosum* L. (2*n* = 16) and *Allium schoenoprasum* L. (2*n* = 16) were studied using RAPD. For this purpose, (i) DNA was isolated as per Greenwood et al.[28] for the removal of polysaccharides, followed by Nucleon Phytopure kit (Amersham Biosciences Corp.) and (ii) RAPD carried out (conditions: 1 cycle for 9 min at 94^{0}C, 45 cycles of 1 min at 94^{0}C, 1 min at 45^{0}C, 2 min at 72^{0}C and 1 cycle for 5 min at 72^{0}C, using a Program

Temp Control System PC-808, Astec Co. Ltd.). The results confirmed the hybridity[29].

Use of RAPD for assessing genetic diversity among cultivars

RAPD markers were also used to estimate genetic diversity among 24 cultivars of short-day onions. For this purpose, (i) total genomic DNA was extracted and subjected to RAPD analysis using arbitrarily 90 decamer primers. Of these primers, (i) only 15 selected primers yielded 137 bands, 91.2% of which were polymorphic and (ii) none produced an unique banding pattern for each cultivar. RAPD analysis (a) grouped 24 onion cultivars into two major clusters, from the northern region and southern region of India, (b) showed high diversity among the selected onion cultivars and (c) indicated the potential of markers for identification and maintenance of onion germplasm for crop improvement[30].

Advantages of RAPD over RFLP

Even though RFLP marker system is quicker to crosses, it is still relatively time-consuming and labor-intensive compared to PCR, which is significantly quicker and cheaper method of evaluating DNA polymorphism[16].

Preparation of genetic map of onion

A low-density genetic map of morphological markers, RAPD and RFLP was developed as a tool for (i) studying the genome organization of onion and (ii) its improvement. For example, (a) a mapping population of 58 F3 families was produced from a single F1 plant from the cross of two partially in-bred lines (Brigham Yellow Globe *15-23* and *Alisa Craig 43*) and (b) segregations (14 RAPDs, 110 RFLPs) were established for restoring male fertility in sterile cytoplasm and complementary light-red bulb colour[31].

Use of RAPD and morphological markers for assessing genetic diversity among Indian cultivars

Field data involved evaluation of 14 cultivars for morphological characters such as (i) plant height (cm), (ii) number of leaves, (iii) bulb weight (g), (iv) bulb diameter (cm), (v) Total Soluble Solids (%), (vi) bolting (%), (vii) doubles (%), (viii) pungency (ppm of pyruvic acid) and (ix) yield (tons/acre), using standard statistical procedure. On the basis of mean performance of these cultivars, their clustering was undertaken, which had grouped them into five clusters. RAPD markers were also used to estimate genetic diversity among these 14 cultivars of short-day onions. For this purpose, total genomic DNA was extracted using modified mini-prep protocol of Vorh et al.[32], with ease and cost effectiveness. RAPD analysis was performed using decamer primers (Figure 1).

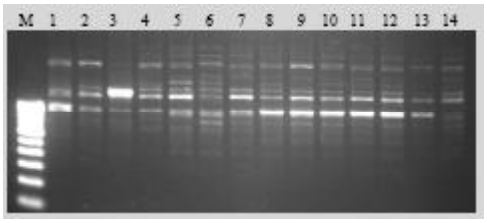

Figure 1: Amplification RAPD profiles of 14 onion cultivars with primer OPC-09(M = 100 bp DNA ladder (Genei, Bangalore), 1= JV-7; 2=JV-12; 3 =JV-16; 4=Phule safed; 5 = Arka kirtiman; 6 = Punjab white; 7 = Agrifound white; 8 = Udaipur-102; 9=Arka Pitambar; 10=Pusa white flat; 11= Pusa white round; 12=Gujarat Local; 13 = ARS-1; 14 = ARL-2.)

The results showed that (i) 31 primers yielded 77.2% polymorphic bands and (ii) none produced an unique banding pattern for each cultivar. RAPD analysis grouped 14 onion cultivars into four clusters, one cluster with cultivars of exotic origin and other three included cultivars of Indian origin. Comparison of clustering based on RAPD as well as field performance indicated (a) high diversity among the onion cultivars selected, (b) maximum divergence among the exotic and Indian cultivars and (c) potential of RAPD markers for identification/maintenance of onion germplasm for onion improvement[33, 34].

CONCLUSIONS

Genus *Allium* contains more than 750 species. RAPD is found to be highly useful in resolving complications arising over origin and interrelations among these species. It is also found to be of use in order to confirm hybrid status of inter-specific hybrids. RAPD in conjugation with other molecular/ morphological approaches is found to be highly useful to assess genetic diversity of onion. Further, it is used to confirm integrity of released hybrids and varieties. In combination with RFLP, RAPD is found out to be highly useful in genetic mapping studies.

Minireview

REFERENCES

[1] G.R.Fenwick, A.B.Hanley, J.L.Brewster, H.D. Rabinowitch; 'Onions and Allied Crops, III, Biochemistry, Food Science and Minor crops', CRC Press, Boca Raton, Florida, 17-31 **(1990a).**

[2] G.R.Fenwick, A.B.Hanley, J.L.Brewster, H.D. Rabinowitch; 'Onions and Allied Crops, III, Biochemistry, Food Science and Minor crops', CRC Press, Boca Raton, Florida, 73-91 **(1990b)**.

[3] H.A.Jones, L.K. Mann; 'Onions and their Allies', Leonard Hill, London, **(1963)**.

[4] A.Takhtajan; 'Diversity and Classification of flowering plants', Columbia University Press, New York, **(1997)**.

[5] J.G.K.Williams, A.R.Kubelik, K.J.Livak, J.A. Rafalski, S.V.Tingey; Nucl.Acid Res., **18**, 6531-6535 **(1990)**.

[6] J.Welsh, M.McClelland; Nucl. Acid Res., **19**, 303-306 **(1990)**.

[7] J.G.K.Williams, J.A.Rafalski, S.V.Tingey; Meth. Enzymol., **218**, 705-740 **(1993)**.

[8] K.Edwards, C.Johnstone, C.Thompson; Nucl.Acid Res., **19**, 1349-1350 **(1991)**.

[9] P.Arus, J.Moreno-Gonzalez, M.D.Hayward, N.O. Bosemark, I.Rmagosa; 'Plant Breeding: Principles and Practices', 314-331 **(1993)**.

[10] M.D.Bennett, J.B.Smith; Phil.Trans.Royal Soc., London, Series, **B274**, 227-274 **(1976)**.

[11] S.D.Tanksley, L.Miller, A.Paterson, R.Bernatzky, J.P.Gustafson, R.Appels Eds. 'Chromosome structure and function', Plennum Press, New York, 157-173 **(1987)**.

[12] S.E.Wilkie, P.G.Isaac, R.J.Slater; Theor.Appl. Genet., **86**, 497-504 **(1993)**.

[13] N.Friesen, M.Klaas; Genet.Res.Crop.Evol., **45**, 511-523 **(1998)**.

[14] A.D.Wolfe, A.Liston, D.E.Soltis, P.S.Soltis, J.J. Doyle; 'Molecular Systematics of Plants, II. DNA Sequencing', Kluwer Academic Publishers, Boston, Mass, 43-86 **(1998)**.

[15] B.Champion, B.Bohanec, B.Javornik; Theor.Appl. Genet., **91**, 598-602 **(1995)**.

[16] M.J.Havey; Theor.Appl.Genet., **90**, 263-268 **(1995)**.

[17] K.F.Bradley, M.A.Rieger, G.G.Collins; Australian J. Exptl.Agri., **36**, 613-618 **(1996)**.

[18] M.A.Al-Zahim, B.V.Ford-lloyd, H.J.Newbury; Plant Cell Reports, **18**, 473-477 **(1999)**.

[19] J.Puizina, B.Javornik, B.Bohanec, D.M.Schweizer, J.Maluszynska, D.Papes; Genome, **42**, 1208-1216 **(1999)**.

[20] R.D.Henfrey, R.J.Slater, J.M.Walker; 'Methods in Molecular Biology', Humana Press, New Jersey, 447-452 **(1988).**

[21] M.A.Saghai-Maroof, K.M.Soliman, R.A.Jorgensen, R.W.Allard; Proc.Natl.Acad.Sci., USA, **81**, 8014-8018 **(1984)**.

[22] J.Sambrook, E.F.Fritsch, T.Maniatis; 'Molecular Cloning: A Laboratory Manual', Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA **(1989)**.

[23] M.J.Bradeen, M.J.Havey; J.Amer.Soc.Horti.Sci., **120**, 752-758 **(1995)**.

[24] M.Dennequin, O.Panaud, T.Robert, A.Ricroch; Heredity, **78**, 403-409 **(1997)**.

[25] N.Friesen, S.Pollner, K.Bachmann, F.R.Blattner; Amer.J.Bot., **86**, 554-562 **(1999)**.

[26] N.S.Arifin, Y.Ozaki, H.Okubo; Euphytica, **111**, 23-31 **(2000)**.

[27] J.Storsberg, H.Schulz, M.Keusgen, F.Tannous, K.J.Dehmer, E.R.J.Keller; J.Agric.Food Chem., **52**, 5499-5505 **(2004)**.

[28] M.S.Greenwood, C.A.Hopper, K.W.Hutchison; Plant Physiol., **90**, 406-412 **(1989)**.

[29] M.Umehara, T.Sueyoshi, K.Shimomura, M.Iwai, M. Shigyo, K.Hirashima, T.Nakahara; Euphytica, **148**, 295-301 **(2006)**.

[30] S.Kutty, V.Gowda, A.Lalitha; J.Horti.Sci. Biotech., **81**, 774-777 **(2006)**.

[31] J.J.King, J.M.Bradeen, M.J.Havey; J.Amer.Soc. Horti.Sci., **123**, 1034-1037 **(1998)**.

[32] B.Vorh, L.Harrengt, A.Chandelier, G.Mergeai, P.Dujardin; Plant Breed., **115**, 205-206 **(1996)**.

[33] G.G.Adsul; Genetic diversity analysis of some onion germplasm lines (Allium cepa L.). Ph.D. thesis, North Maharashtra University, Jalgaon, **(2009)**.

[34] G.G.Adsul, D.G.Patil, A.V.Dhake, U.B.Pandey, R.M.Kothari; Adv.Plant Sci., (In press).