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Physical localization of highly nutritional protein coding *AmA1* gene in amaranths through molecular cytogenetic tools

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Abstract — A highly nutritional protein coding gene *AmA1* of *Amaranthus hypochondriacus* was searched in different species of *Amaranthus* by using various molecular biological techniques. PCR, Southern hybridization, primed *in situ* labeling (PRINS) and Cycling-primed *in situ* labeling (C-PRINS) confirmed the presence of AmA1 coding region only in *Amaranthus hypochondriacus.* The *AmA1* gene was successfully localized on interphase nuclei and also on the metaphase chromosomes. The single copy, unique *AmA1* gene was found to be present in the subtelomeric region of two homologous chromosomes in the group of 2-2.2 µm in length. In the present study, a rapid and reliable method for physical localization of single gene on plant chromosome was described and both the methods for *in situ* localization i.e. PRINS and C-PRINS were compared.

Key words: *Amaranthus*, *AmA1* gene*,* cycling-primed *in situ* labeling (C-PRINS), primed *in situ* labeling (PRINS).

INTRODUCTION

Amaranths are the most important group of plants in Amaranthaceae containing many leafy vegetables and grain types. The genus *Amaranthus* is widely distributed throughout the worlds of temperate and tropical regions. Traditionally these native American crops have been utilized for its food value by people from Mexico to Peru to Nepal long before any nutritional analyses had been conducted. Though both the grain and leafy biotypes of *Amaranthus* species are very popular in India, grain amaranths have gained much importance for its high protein content (17-19% of seed dry weight) as compared to other traditional crops having an average of ~10% protein (Larkins 1981). Since past few years amaranths have received renewed attention as alternative field crops (Bressani *et al.* 1987a; b; Tucker 1986) mainly due to its essential amino acids such as lysine, tryptophan and sulfur containing amino acids rich protein (Senft 1980). A highly nutritional, 35 kDa protein AmA1 has been reported from *Amaranthus hypochondriacus* corresponding closely to that of World Health Organization recommended protein standard for optimum human nutrition (Raina and Datta 1992). There are several other popularly edible grain and leafy vegetables of amaranth, which had not been studied for identification of this 35 kDa AmA1 protein encoding gene. Despite the increasing attention there is neither a genetic nor any cytological linkage map available in case of amaranths. In this present study, our main intention was to investigate the presence of this highly nutritional protein coding *AmA1* gene in other grain and leafy vegetables of amaranths and also to localize the *AmA1* gene physically on chromosome.

Attempts to localize specific nucleotide sequences on chromosomes were however quite limited in plants. Moreover it had been difficult to identify chromosomes objectively by morphological means in plants having small chromosomes such as amaranths measuring 1.1-2.8 µm at mitotic metaphase. Most popular non isotopic labeling of nucleic acids, fluorescence *in situ* hybridization (FISH) has been extensively used to visualize DNA sequences in interphase nuclei (Dong and Jiang 1998), mitotic and meiotic chromosomes (Nenno *et al.* 1994; Pedersen *et al.* 1995) as well as on extended DNA fibres (De Jong *et al.* 1999) and bacterial artificial chromosomes (Jiang *et al.* 1995). Plant cytogeneticists used conventional *in*

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situ hybridization technique to distinguish between different genomes and for physical mapping of repeated sequences (HESLOP-HARRISON 1991). The detection of low copy sequences, such as the secalin gene cluster in rye (GUSTAFSON *et al.* 1990) and a number of unique sequences have also been reported in plants (AMBROS *et al.* 1986; SIMPSON *et al.* 1988; GUSTAFSON and DILLÉ 1992). However efforts to detect reliably unique sequences on plant chromosomes by using labeled DNA probes have a low success rate. It has been reported that localization of DNA sequences shorter than 10 kb on plant chromosomes using ISH or FISH is still very difficult (JIANG and GILL 1994). The occurrence of cell wall fragments and cellular debris reduce the access of DNA probes to the target sites on plant chromosomes decreasing the sensitivity of *in situ* detection. Furthermore detection of certain sequences may be hindered by the presence of highly condensed chromatin, as seen in human and plant chromosome preparations (HOOVERS *et al.* 1992; SHEN *et al.* 1987). Alternative to FISH, primed *in situ* labeling (PRINS) and Cycling-PRINS has several advantages. For PRINS, short oligonucleotide primers can be designed and synthesized with a minimum of sequence information, which hybridize to homologous targets within densely structured chromatin both easier and faster than the larger probes usually used for FISH. The use of an oligonucleotide as annealed primer also allows better penetration of debris and condensed chromatin, thus increasing the efficiency of hybridization and sensitivity of detection. In C-PRINS, sequence of thermal cycles analogous to polymerase chain reaction are involved (GOSDEN *et al.* 1991; TERKELSEN *et al.* 1993a; b) in which 20% increase in labeled DNA after each cycle of C-PRINS has been reported (TERKELSEN *et al.* 1993a). Because of its speed and sensitivity, the number of application of PRINS and C-PRINS in human cytogenetics is rapidly increasing (KOCH *et al.* 1995a; b; THERKELSEN *et al.* 1995; PELLESTOR *et al.* 1996). However C-PRINS was used to detect DNA sequences shorter than 2 kb on human chromosomes (GOSDEN and LAWSON 1994a; b). Direct PRINS and C-PRINS have been reported for the rapid physical mapping of several types of repetitive DNA sequences on the mitotic chromosomes of *Vicia faba*, *Pisum sativum*, and *Secale cereale* (KUBALÁKOVÁ *et al.* 1997). Both the techniques have already been used successfully for the detection of unique transgene on rice chromosomes (SAHA *et al.* 2006).

In this communication an attempt has been made to standardize a protocol for reliable and rapid physical localization of the unique gene on plant chromosomes. Moreover to study the distribution of *AmA1* gene in other amaranths, PCR, Southern blot analyses and physical localization of gene on chromosome by PRINS and C-PRINS techniques have been employed. In addition, potential applications of both the techniques, PRINS and C-PRINS were compared.

MATERIALS AND METHODS

Plant Materials - Plant materials used in this study are listed in Table 1. Seeds of three grain amaranths, *A. hypochondriacus*, *A. caudatus* and *A. cruentus* of different accessions were obtained from National Bureau of Plant Genetic Research (NBPGR), Simla, India and seven leafy vegetables of amaranths, *A. gangeticus* (syn. *tricolor*), *A. paniculatus, A. viridis, A. blitum, A. lividus, A. tristis and A. polygamous* were collected from different localities of West Bengal and other parts of India and were identified by Botanical Survey of India (BSI), Kolkata, India.

cDNA of AmA1 - E. coli strain DH-5α harboring the plasmid pTZ18U containing *AmA1* cDNA (pAmA1.3) was obtained from National Institute for Plant Genome Research (NIPGR), Jawaharlal Nehru University, New Delhi, India.

Isolation of genomic DNA - Genomic DNA was isolated from young leaves of both leafy vegetables and grain amaranths using a protocol modified from DELLAPORTA *et al.* (1983). Briefly tissues were ground to fine powder and extracted for 20 min at 65° C using extraction buffer (50 mM Tris-Cl pH 8.0, 10 mM EDTA pH 8.0, 100 mM NaCl, 1% SDS and 10 mM 2-mercaptoethanol). The supernatant was extracted twice with phenol: chloroform (1:1), 1/10th volume of 3 M ammonium acetate and equal volume of pre-chilled isopropanol were added, stored at -20° C for overnight and centrifuged at 10000 *g* for 10 min. The pellet was suspended in TE buffer (10 mM Tris and 1 mM EDTA pH 8.0) after washing with 70% ethanol. To remove co-precipitated RNA, RNase treatment was done. Polyethylene glycol (PEG 8000) treatment was done to remove proteins and polysaccharides from DNA following DIXIT (1998). Concentration of DNA sample was determined by comparing band intensity with known concentration of lambda DNA digest after 0.8% agarose gel electrophoresis and ethidium bromide staining and also by spectrophotometric measurement of the OD reading at 260 and 280 nm against TE buffer in Beckman DU 530 spectrophotometer.

PCR amplification of AmA1 - The AmA1 coding region was amplified by PCR using forward primer F51 (5'-CACCATGGCGGGATTACCAGTG-3') and reverse primer R1044 (5'-CAAGGAAGAACCCTCTTGTTTCC-3') as reported by CHAKRABORTY *et al.* (2000). PCR amplification was carried out with 20 pmol each of *AmA1* gene specific primers F51 and R1044, 0.3 mM dNTP mix (MBI Fermentus, Hanover, MD, USA), 2.5 µl of 10 X PCR buffer (MBI Fermentus), 2.5 U *Taq* DNA polymerase (MBI Fermentus), 2 mM $MgCl_2$ (MBI Fermentus) and 1 µg template DNA in 25 µl reaction volume. Amplification reactions were performed in a PerkinElmer model 2400 thermocycler (Perkin Elmer, Waltham, MA, USA) as follows: initial denaturation at 94° C for 5 min followed by 30 cycles of 94° C for 1 min, 60° C for 1 min and 72° C for 1min and final elongation at 72° C for 7 min. The amplified products were separated by 1% agarose gel electrophoresis (AGE), stained with ethidium bromide (Sigma, USA) and visualized under UV illumination.

Southern blot hybridization - Southern blot analysis was done according to the methods of SAMBROOK *et al.* (1989) with slight modifications. Approximately 10 µg of genomic DNA from all the species of *Amaranthus* (Table 1) were digested with *Eco*RI (Roche Diagnostics, GmbH, Mannheim, Germany) and DNA from *A. hypochondriacus* was digested with *Bam*HI (Roche), *Bgl*II (Roche), *Hind*III (Roche) and *Eco*RI respectively, followed by separation in 0.8% (w/v) agarose gel. After complete denaturation and renaturation, the gel was blotted onto positively charged Hybond-N^+ nylon membrane (Amersham Biosciences, Buckinghamshire, UK).

The 1.2 kb *AmA1* sequence was digested out from pAmA1.3 plasmid using *Eco*RI restriction enzyme according to the map provided by CHAKRABORTY *et al.* (2000), gel was eluted using Qiagen gel elution kit (Qiagen, Valencia, CA, USA) and labeled with [α-32P] dCTP (Perkin Elmer) using "Ready Prime" random labeling system (Amersham Biosciences). The membranes were hybridized with radiolabeled *AmA1* gene probe for overnight at 68° C, followed by washing twice in buffer 1 (2 X SSC and 0.1% SDS) at room temperature and twice in buffer 2 (0.1 X SSC and 0.1% SDS) at 68° C for 30 min per wash. The membranes were then exposed to Kodak X-ray film for 7 days at - 80° C and thereafter the films were developed.

Chromosome preparation and karyogram analyses - About 2–4 mm long root tips were excised and put in ice cold water for 20–24 h, followed by fixation in ethanol:acetic acid (3:1, v/v) at 14–18° C for overnight. Fixed root tips were washed thoroughly with 0.01 M citrate buffer (sodium citrate/citric acid, pH 4.8) and digested with 1% (w/v) cellulase (Sigma) for 1 h and 2% (w/v) pectinase (Sigma) for 30 min at 37° C. After careful removal of enzymes from the softened material by washing with distilled water for 30 min with three changes each after 10 min, the root tips were transferred on Amplislides. Finally, the treated root tips were squashed, air dried and stored at -20° C. Karyogram of metaphase chromosome was performed using IKAROS Metasystem (v.3.4.0) karyotyping software.

Primed in situ DNA labeling and cycling-primed in situ labeling - The procedures of primed *in situ* DNA labeling (PRINS) and cycling-primed *in situ* labeling (C-PRINS) were modified from the method of ABBO *et al.* (1993) and KUBALÁKOVÁ *et al.* (2001). Slides without any pretreatment were used directly for reaction and/or alternatively, the slides were washed three times in 2 X SSC for 5 min at room temperature followed by an RNase (50 µg ml^{-1}, Sigma) treatment in reaction buffer (1 mM Tris–HCl, 1.5 mM NaCl, pH 7.5) at 37° C for 60 min and proteinaseK (2 µg ml^{-1}, Sigma) treatment at room temperature for 1 min or gradual dehydration with 70-90% ethanol. After pretreatment the slides were rinsed in 1 X PCR buffer (10 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$) for 5 min and used for reaction. For direct PRINS, the labeling reactions were performed in 50 µl volume containing 1 X PCR buffer (Ampli Taq, Applied Biosystem), 2.5 mM $MgCl_2$, 200 µM of each primers, 100 µM of each dATP, dCTP, dGTP, 17 µM of dTTP and 8 µM of fluorescein-11-dUTP (Roche) and 3 U of Amplitaq gold DNA polymerase. Reaction mixture was sealed under Amplicover discs with Amplicover clips. Thermal cycle comprised of 18 min at 95° C to activate the Amplitaq gold DNA polymerase, then 94° C for 2 min, 60° C for 15 min and extended at 72° C for 45 min. Reaction mixture for C-PRINS was same as PRINS except 2 mM $MgCl_2$, 34 µM of dTTP, 2 µM of fuorescein-11-dUTP and 5 U of Amplitaq gold DNA polymerase were used. C-PRINS reaction was carried out following an initial cycle of 18 min at 95° C, 1 min at 94° C, 5 min at 60° C, 10 min at 72° C, followed by 30 cycles each of 1 min at 94° C, 1 min at 60° C and 2 min at 72° C with a final extension for 7 min at 72° C. The reactions were carried out using GeneAmp *in situ* PCR system 1000 (Applied Biosystems, Foster City, CA, USA) and the experiments were repeated at least thrice to establish the reproducibility of the results.

Chromosome staining, signal detection and fluorescence microscopy - Both PRINS and C-PRINS reactions were terminated with stop buffer (0.5 M NaCl, 0.05 M EDTA, pH 8.0) for 5 min at 65° C and washed with wash buffer (0.1 M maleic acid, 0.15 M NaCl, 0.05% Tween 20, pH 7.5) at room temperature, for 5 min. The cover slips were immediately mounted and counter stained with Vectashield antifade solution (Vector Laboratories, Burlingame, CA, USA) containing 4',6'-diamidino-2-phenylindole (DAPI, 0.2 µg ml^{-1}). Amplislides were examined with a Zeiss Axioskop 2 fuorescence microscope equipped with filter sets for the detection of fluorescein isothiocyanate (FITC) and DAPI, 100 W HBO and HAL illuminator. Filter set 1 and set 9 were used for the visualization of fluorescein and DAPI fuorescence, respectively. Signals were recorded by CCD camera and analyzed by FISH ImagerTM (v.1.0) software.

RESULTS AND DISCUSSION

PCR Analysis - For identification of *AmA1* gene in other amaranths, PCR was performed in all the plant materials as listed in Table 1 such as leafy vegetables, *A. gangeticus* (syn. *tricolor*), *A. paniculatus, A. viridis, A. blitum, A. lividus, A. tristis and A. polygamous* and all the grain amaranths, *A. hypochondriacus*, *A. caudatus* and *A cruentus* using the *AmA1* specific primer pairs. A single band was detected in all the accessions of *A. hypochondriacus* by PCR amplification (Fig. 1), but no other leafy vegetables or grain amaranths produced the same band. To check the reproducibility, the PCR reaction was repeated several times. The *AmA1* specific primer pair consistently produced the same sized band only in *A. hypochondriacus,* suggesting the specificity of *AmA1* coding nucleotide sequence in *A. hypochondriacus*.

Southern hybridization - Southern hybridization was done to confirm the occurrence of *AmA1* among all the amaranths of the present study (Table 1). After hybridization of the radiolabelled *AmA1* specific probe with the genomic DNA digested with *Eco*RI, single hybridized band was observed in the lanes of *A. hypochondriacus* (Fig. 2a). Other amaranths of both the biotypes did not produce any hybridization signals. Absence of any non-specific band confirmed the absence of any homologous sequence in other species of *Amaranthus* of this study. For further confirmation of the presence of *AmA1* in *A. hypochondriacus*, *Bam*HI, *Bgl*II, *Hind*III and *Eco*RI digested DNA of different accessions of *A. hypochondriacus* were separately hybridized with radiolabeled *AmA1* gene specific probe. In all cases it produced strong signals (Fig. 2b) confirming that highly nutritional protein coding single gene *AmA1* is present only in *A. hypochondriacus*.

PRINS and C-PRINS detection - PRINS and C-PRINS reactions were performed in all the materials as listed in Table 1. Previously, the specificity of the primers had been established by PCR analysis with an annealing step of 60° C. In both the *in situ* detection methods, optimized protocols resulted in clear signals only in *A. hypochondriacus* with negligible non specific labeling (Fig. 3), but strong signals were obtained after C-PRINS

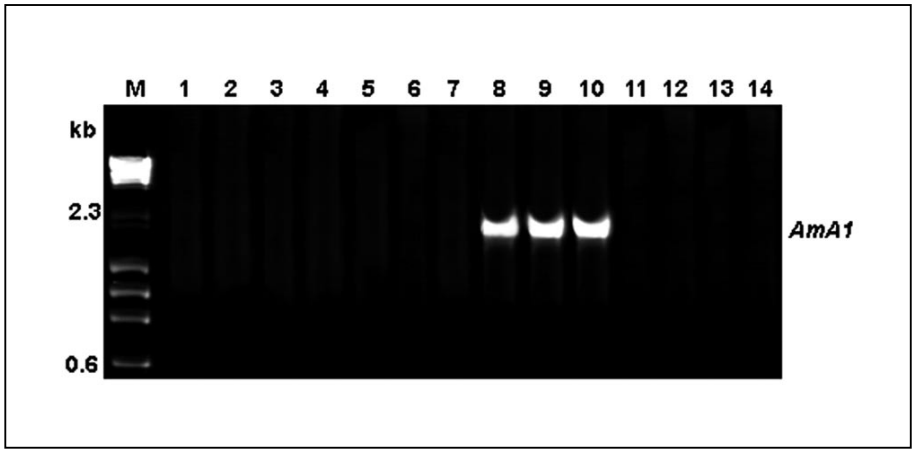

Fig. 1 — PCR amplification of genomic DNA with *AmA1* specific primer producing single band of *AmA1* gene. Lane M: λ DNA *Hind* III digest and ΦX 174 DNA *Hae* III digest marker. Lane 1 -14: AG1, AP1, AV1, AB, AL, ATR, APL, AH1, AH2, AH3, ACD1, ACD2, ACR1 and ACR2 in a sequence as mentioned in Table 1.

Table 1 — Members of amaranths used in the present study.

Biotype	Taxon	Population Code or Accession Number	Source	Chromosome Number (2n)
Vegetative	*Amaranthus gangeticus*	AG1	West Bengal, India	32
	Amaranthus paniculatus	AP1	West Bengal, India	32
	Amaranthus viridis	AV1	West Bengal, India	32
	Amaranthus blitum	AB	West Bengal, India	32
	Amaranthus lividus	AL	Tamil Nadu, India	32
	Amaranthus tristis	ATR	Tamil Nadu, India	32
	Amaranthus polygamous	APL	West Bengal, India	32
Grain type	*Amaranthus hypochondriacus*	AH1/ IC17930	NBPGR, Simla, India	32
	Amaranthus hypochondriacus	AH2/ IC17936	NBPGR, Simla, India	32
	Amaranthus hypochondriacus	AH3/ IC17938	NBPGR, Simla, India	32
	Amaranthus caudatus	ACD1/ IC381185	NBPGR, Simla, India	32
	Amaranthus caudatus	ACD2/ IC381200	NBPGR, Simla, India	32
	Amaranthus cruentus	ACR1/ EC277971	NBPGR, Simla, India	32
	Amaranthus cruentus	ACR2/ EC321563	NBPGR, Simla, India	32

in both interphase nuclei (Fig. 3b) and early metaphase chromosomes (Fig. 3d). Although the images were captured by CCD camera and processed by image analysis software, they were clearly visible through the microscope.

FITC fluorescence revealed specific labeling of a single pair of chromosomes in *A. hypochondriacus* of all the accessions (Fig. 3c, d). It suggests that single copy, unique *AmA1* gene is present in two homologous chromosomes of *A. hypochon-*

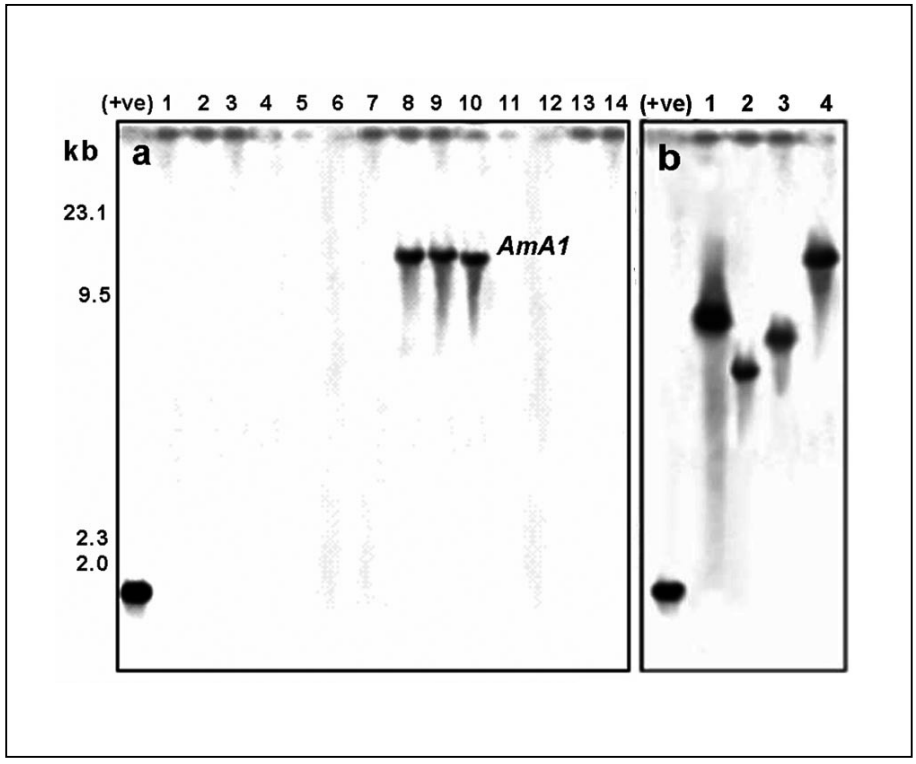

Fig. 2 — Southern blot analysis. **a** DNA was digested with *Eco*RI. Lane 1 -14: AG1, AP1, AV1, AB, AL, ATR, APL, AH1, AH2, AH3, ACD1, ACD2, ACR1 and ACR2 in a sequence as mentioned in Table 1; **b** DNA of *A. hypochondriacus* (IC17930) was digested with *Bam*HI (lane1), *Bgl*II (lane2), *Hind*III (lane 3) and *Eco*RI (lane 4) respectively. Lane (+ve): 1.2 kb *AmA1* gene fragment as positive control.

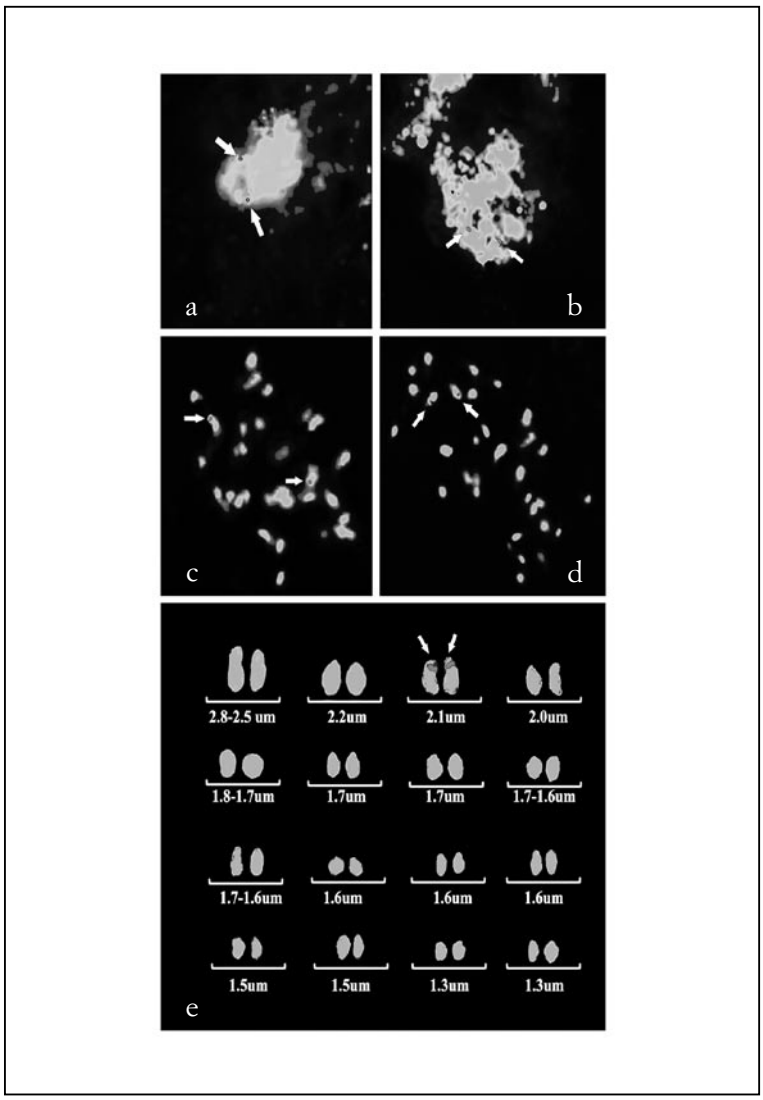

Fig. 3 — Localization of *AmA1* sequence in *A. hypochondriacus*. **a** on interphase nuclei by PRINS (ca. x 1000); **b** on interphase nuclei by C-PRINS (ca. x 1000); **c** on early metaphase chromosome by PRINS (ca. x 1000); **d** on early metaphase chromosome by C-PRINS (ca. x 1000); **e** Karyogram after localization of *AmA1* gene by C-PRINS. Arrows on the figures indicate the strong hybridization signals (yellow-green colour spot) obtained after incorporation of fluorescein-11dUTP. Chromosomes are counterstained with DAPI (blue colour).

driacus. After primer annealing and extension, two distinct sites were also detected on the interphase nuclei (Fig. 3a, b). The exact identification of chromosomes bearing *AmA1* gene could not be done because of its very small size with no distinct difference in chromosome morphology. However detailed chromosome morphology of *Amaranthus hypochondriacus* was studied through IKAROS software (Fig. 3e). The length of chromosomes bearing *AmA1* gene ranges from 2-2.2 µm. As the chromosomes were in little early metaphase, determination of the exact length of chromosomes bearing *AmA1* gene was not possible. Although it can be said that chromosomes bearing *AmA1* come in the group of 2-2.2 µm long chromosomes. In metaphase plate, the FITC signals were found to be present at the sub-telomeric region of homologous chromosomes. The signal position at the distal part of chromosomes may be due to the fact that distal regions are the most decondensed part of cereal chromosomes and contains a large number of actively transcribed genes that are active during the cell cycle of cells that divide rapidly (JIN *et al.* 2002).

According to MACAS *et al.* (1995) and KUBALÁKOVÁ *et al.* (1997), indirect PRINS increases signals as well as non specific labeling so in our study direct PRINS was used to detect the gene on chromosome. A potential of indirect C-PRINS for detection of single copy DNA sequences in plant chromosomes was demonstrated by ZHU *et al.* (1995) and SHI *et al.* (1996) who localized single copy DNA sequences as short as 1 kb in soybean. In comparison to indirect C-PRINS followed by MUKAI and APPELS (1996), the method for direct C-PRINS is faster and more sensitive (KUBALÁKOVÁ *et al.* 1997). KUBALÁKOVÁ *et al.* (2001) demonstrated the usefulness of direct PRINS and C-PRINS for localization of various types of repeated DNA sequences in plant chromosomes. In our study we also noticed better signals after C-PRINS in comparison to PRINS as observed by KUBALÁKOVÁ *et al.* (2001).

The specificity and sensitivity of PRINS and C-PRINS depends on a number of factors (KUBALÁKOVÁ *et al.* 1997; 1998; LYSÁK *et al.* 1999). The quality and age of chromosome preparation were extremely important. In our investigation, signals were best detected on fresh slides (after one or two days of squash preparation). Though KUBALÁKOVÁ *et al.* (1997) observed telomeric sequences best on 2-4 week old slides, other sequences were best detected on 1 week old slides. According to them aging may decrease non specific labeling on chromosomes. This observation differs from KOCH *et al.* (1995b) who found that the background for human chromosomes increased with the age of slides. RNase treatment is another factor which helped to reduce non specific labeling, but at the same time it resulted into less intense signal. Similar observation was also reported by KUBALÁKOVÁ *et al.* (1997). Though GODSEN and LAWSON (1994b) recommended a passage through ethanol series, it produced negatively influenced chromosome morphology, decreased signal intensity and caused additional non-specific labeling around the chromosomes. So we did not follow this treatment further. In our study, slides treated with RNase and proteinase K produced best detectable signal without non specific labeling. *In situ* detection of low- and single-copy DNA sequences has proved to be difficult for plant chromosomes. Probes above a certain size may not efficiently penetrate cell wall fragments, cellular debris and condensed chromatin to reach their target sites. Alternatively the use of short oligonucleotides as the hybridizing reagent could increase sensitivity by improving penetration of the probe DNA to target sequences. Although C-PRINS resulted in intense signal than PRINS, both the techniques confirmed physical localization of *AmA1* gene on interphase nuclei and chromosomes. Moreover screening of specific gene in different taxa can be done rapidly by PRINS and C-PRINS than any other *in situ* hybridization methods.

In this study it was observed by PCR using *AmA1* specific primers that *AmA1* gene is present only in *A. hypochondriacus*. Further the same observation was confirmed by Southern hybridization using *AmA1* specific probe. No other popularly edible grain or leafy amaranths contain this highly nutritional protein coding *AmA1* gene. Both PRINS and C-PRINS successfully localized the single gene *AmA1* on interphase nuclei and chromosomes of all the accessions of *A. hypochondriacus.* In conclusion, these *in situ* hybridization techniques, PRINS and C-PRINS are simple, fast and efficient methods in reliable physical localization of unique gene on plant chromosome.

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REFERENCES

ABBO S., MILLER T.E. and KING I.P., 1993 — *Primed-induced in situ hybridization to plant chromosomes.* Genome, 36: 815-817.

AMBROS P.F., MATZKE M.A. and MATZKE A.J.M., 1986 — *Detection of 17-kb unique sequence (T-DNA) in plant chromosomes by in situ hybridization.* Chromosoma, 94: 11-18.

BRESSANI R., GONZALES J.M., ZUNIGA J., BRAUNER M. and ELIAS L.G., 1987a — *Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species.* Journal of the Science of Food and Agriculture, 38: 347-356.

BRESSANI R., GONZALES J.M., ZUNIGA J., BRAUNER M. and ELIAS L.G., 1987b — *Effect of fertilizer application on the field, protein and fat content and protein quality of raw and cooked grain of three amaranth species*. Qualitus Plantrum, 37(1): 59-67.

CHAKRABORTY S., CHAKRABORTY N. and DATTA A., 2000 — *Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene for Amaranthus hypochondriacus*. Proceedings of the National Academy of Science of the United States of America, 97 (7): 3724-3729.

DE JONG J.H., FRANSZ P. and ZABEL P., 1999 — *High resolution FISH in plants- techniques and applications.* Trends in Plant Science, 4: 258-263.

DELLAPORTA S.L., WOOD J. and HICKS J.B., 1983 — *A plant DNA mini-preparation: version II.,* Plant Molecular Biology Reporter, 1:19–21.

DIXIT A., 1998 — *A simple and rapid procedure for isolation of Amaranthus DNA suitable for fingerprint analysis*. Plant Molecular Biology Reporter, 16: 1-8.

DONG F. and JIANG J., 1998 — *Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells*. Chromosome Research, 6: 551-558.

GOSDEN J., HANRATTY D., STARLING J., FANTES J., MITCHELL A. and PORTEOUS D., 1991 — *Oligonucleotide-primed in situ DNA synthesis (PRINS): a method for chromosome mapping, banding, and investigation of sequence organization*. Cytogenetic and Genome Research, 57: 100-104.

GOSDEN J.R. and LAWSON D., 1994 a — *Oligonucleotide-primed in situ DNA synthesis (Prins)- an alternative to in situ hybridization for gene mapping and the investigation of genome organization*. In: J.R. Gosden (Ed), "Chromosome analysis protocols", pp. 323-333. Humana Press Inc, Totowa, New Jersy.

GOSDEN J.R. and LAWSON D., 1994 b — *Rapid chromosome identification by oligonucleotide-primed in situ DNA synthesis (PRINS).* Human Molecular Genetics, 3: 931-936.

GUSTAFSON J.P., BUTLER E. and MCINTYRE C.L., 1990 — *Physical mapping of a low-copy DNA sequence in rye {Secale cereale L.}.* Proceedings of the National Academy of Science of the United States of America, 87: 1899-1902.

GUSTAFSON J.P. and DILLÉ J.E., 1992 — *Chromosome location of Oryza sativa recombination linkage groups.* Proceedings of the National Academy of Science of the United States of America, 89: 8646-8650.

HESLOP-HARRISON J.S., 1991 — *The molecular cytogenetics of plants*. Journal of Cell Science, 100: 15-21.

HOOVERS J.M.N., MANNENS M., JOHN R., BLEIK J., VAN HEYNINGEN V., PORTEOUS D.J., LESCHOT N.J., WESTERVELD A. and LITTLE P.F.R., 1992 — *High resolution localization of 69 potential human zinc finger protein genes: A number are clustered.* Genomics, 12: 254-263.

JIANG J. and GILL B.S., 1994 — *Non-isotopic in situ hybridization and plant genome mapping: the first ten years*. Genome, 37: 717-725.

JIANG J., GILL B.S., WANG G.L., RONALD P.C. and WARD D.C., 1995 — *Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes*. Proceedings of the National Academy of Science of the United States of America, 92: 4487-4491.

JIN W-W., LI Z-Y., FANG Q., ALTOSAAR I., LIU L-H. and SONG Y-C., 2002 — *Fluorescence in situ hybridization analysis of alien genes in Agrobacterium – mediated Cry1A(b)-transformed rice*. Annals of Botany, 90: 31-36.

KOCH J., HINDKJÆR J., KØLVRAA S., NYGAARD M. and BOLUND L., 1995a — *Primed in situ labelling (PRINS)*. In: "In situ hybridization. Hand books of laboratory methods", pp. 1-19. Springer, Verlag, Berlin.

KOCH J., HINDKJÆR J., KØLVRAA S., NYGAARD M. and BOLUND L., 1995b — *Construction of a panel of chromosome-specific oligonucleotide probes (PRINS-primers) usefull for the identification of individual human chromosomes in situ*. Cytogenetic and Genome Research, 71: 142-147.

KUBALÁKOVÁ M., MACAS J. and DOLŽEL J., 1997 — *Mapping of repeated DNA sequences in plant chromosomes by PRINS and C-PRINS.* Theoretical and Applied Genetics, 94: 758-763.

KUBALÁKOVÁ M., NOUZOVÁ N., DOLEŽELOVA M., MACAS J. and DOLŽEL J., 1998 — *A combined PRINS-FISH technique for simultaneous localization of DNA sequences on plant chromosomes*. Biologia Plantarum, 42: 293-296.

KUBALÁKOVÁ M., VRÁNA J., ČÍHALÍKOVÁ J., LYSÁK M.A. and DOLŽEL J., 2001 — *Localisation of DNA sequences on plant chromosomes using PRINS and C-PRINS*. Methods in Cell Science, 23: 71-82.

LARKINS B.A., 1981 — *The biochemistry of plants*. Academic, New York.

LYSÁK M.A., ČÍHALÍKOVÁ J., KUBALÁKOVÁ M., ŠIMKOVÁ H., KUNZEL G. and DOLŽEL J., 1999 — *Flow karyotyping and sorting of mitotic chromosomes of barley (Hordeum vulgare L.).* Chromosome Research, 7: 431-444.

MACAS J., DOLŽEL J., GUALBERTI G., PICH U., SCHUBERT I. and LUCRETTI S., 1995 — *Primer-induced labeling of pea and field bean chromosomes in situ in suspension*. BioTechniques, 19: 402-408.

MUKAI Y. and APPELS R., 1996 — *Direct chromosome mapping of plant genes by in situ polymerase chain reaction (in situ PCR).* Chromosome Research, 4: 401-403.

NENNO M., SCHUMANN K. and NAGL W., 1994 — *Detection of rRNA and phaseolin genes on polytene chromosomes of Phaseolus coccineus by fluorescence in situ hybridization after pepsin pretreatment*. Genome, 37: 1018-1021.

PEDERSEN C., GIESE H. and LINDE LAURSEN I., 1995 — *Towards an integration of the physical and the genetic chromosome maps of barley by in situ hybridization*. Hereditas, 123: 77-88.

PELLESTOR F., QUENNESSON I., COIGNET L., GIRARDET A., ANDREO B. and CHARLIEU J.P., 1996 — *Direct detection of disomy in human sperm by the PRINS technique*. Human Genetics, 97: 21-25.

RAINA A. and DATTA A., 1992 — *Molecular cloning of a gene encoding a seed specific protein with nutritionally balanced amino acid composition from Amaranthus*. Proceedings of the National Academy of Science of the United States of America, 89: 11774-11778.

SAHA P., MAJUMDER P., DUTTA I., RAY T., ROY S.C. and DAS S., 2006 — *Transgenic rice expressing Allium sativum leaf lectin with enhanced resistance against sap-sucking insect pests*. Planta, 223: 1329-1343.

SAMBROOK J., FRITSCH E.F. and MANIATIS T., 1989 — *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

SENFT J.P., 1980 — *Proc 2nd Amaranth Conf* Rodale, pp. 43-47. Emmaus, PA.

SHEN D.L., WANG Z-F. and WU M., 1987 — *Gene mapping on maize pachytene chromosomes by in situ hybridization.* Chromosoma, 95: 311-314.

SHI L., ZHU T., MORGANTE M., RAFALSKI J.A. and KEIM P., 1996 — *Soybean chromosome painting: a strategy for somatic cytogenetics*. Journal of Heredity, 87: 308-313.

SIMPSON P.R., NEWMAN M-A. and DAVIES D.R., 1988 — *Detection of legumin gene DNA sequences in pea by in situ hybridization.* Chromosoma, 96: 454-458.

TERKELSEN C., KOCH J., KØLVRAA S., HINDKJÆR J., PEDERSEN S. and BOLUND L., 1993a — *Repeated primer in situ labeling: formation and labeling of specific DNA sequences in chromosomes and nuclei*. Cytogenetic and Genome Research, 63: 235-237.

TERKELSEN C., KOCH J., KØLVRAA S., HINDKJÆR J., PEDERSEN S. and BOLUND L., 1993b — *Repeated primer in situ labeling of specific DNA sequences ("PCR-PRINS") in metaphase chromosomal spreads*. Colloquium, 3: 10-12.

THERKELSEN A.J., NIELSEN A., KOCH J., HINDKJÆR J. and KØLVRAA S., 1995 — *Staining of human telomeres with primed in situ labeling (PRINS).* Cytogenetic and Genome Research, 68: 115-118.

TUCKER J.B., 1986 — *Amaranth: the once and future crop.* BioScience, 36: 9-13, 59-60.

ZHU T., SHI L. and KEIM P., 1995 — Detection by in-situ fluorescence of short, single copy sequences of chromosomal DNA. Plant Molecular Biology Reporter, 13: 270-277.

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