MOLECULAR CHARACTERIZATION OF AMARANTHUS RETROFLEXUS RBCL GENE

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ABSTRACT:

People perception is usually influenced by the appearance of the food by colouring. Different foods are associated with different colours by people. Artificial food colouring agent cause human health problems, Carcinogen, Intestinal bleeding, Diarrhoea & excessive menstruation, mainly cause [ADHD] Attention defect hyperactivity disorder in children. Natural colouring cannot cause these problems. In the present study Amaranthus leaf pigment (betacyanin) was used as food colouring agent. Amaranthus is defined as “never-fading flower” in Greek. Several species of Amaranthus are often considered as weeds, people around the world worth Amaranthus as leaf vegetables, cereals and ornamentals.

DNA was extracted from leaf tissue using a modification of the cetyl trimethyl ammonium bromide (cTAB) method. We developed and tested a set of primer for the specific amplification of rbcl gene segments from Amaranthus by PCR. The rbcL gene encodes ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit. The PCR amplification profile was checked on agarose gel and the product size was compared with a size ladder of 100bp. The PCR product was sequenced by Sanger sequencing method. Phylogenetic analysis the sequences were aligned using Clustal W, and the genetic distances were computed according to the Kimura 2-Parameter (K2P) model. The rbcL gene has 700 bp in length and has conserve primer in PCR. DNA bar coding the genus of the sample is Amaranthus. The primer used for the analysis showed similarity to retroflexus species. Thus we can conclude that the given sample might be Amaranthus retroflexus.

Keywords: Amaranthus retroflexus, rbcL gene, PCR.
INTRODUCTION:

Colour is an important characteristic to choose food by people’s. The visual aspect of food products can be an important factor in a person’s decision. Colour thus making the product more attractive and increasing people’s acceptability. For that reason, the use of dye’s and artificial colouring. Artificial colour additives are widely used to enhance the appearance of food and beverages. However, because of concerns about the potential health risk from the consumption of artificial food colourings. Health problems such as physical and mental disorders mainly in children’s, impulsiveness, inattentiveness, DNA damage, chromosomal aberration’s and causing intestinal problems in human. To avoid these problems, the use of natural dyes or natural colouring from the naturally occurring sources such as plants, animals, insects, and minerals. In this way, medicinal plants are used as food additives.

Medicinal plant’s does not produce any human health problems. They are rich in minerals (calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese) and vitamins (vitamin A, vitamin B6, vitamin C, riboflavin and folate) (Gerold Jerz et al., 2007). It will produce the natural colour and act as a food additive. So medicinal plants have much attention in recent times because of their diversity in treating disease and its medicinal properties. Dependence on herbs as medicine for treatment of disease. The increasing awareness towards traditional medicine in human and animal healthcare paved the way to research on efficacy of the herbs used in the treatment of illness. The WHO supports and accepts the traditional medicine as a valuable resource for primary health care (Manila S, 1993). Plants are a source of many potent and powerful drugs used medicinally in different countries (Hashim et al., 2010). A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties (Arora DS et al., 2007).

Amaranthus is a medicinal plant, this plant leaf have a pigment, it is used for food colouring agent. The plant has several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, betalains, B-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins and carotenoids. It also contains amaranthoside, a lignin glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline (Azhar-ul-Haq et al., 2006; Blunden et al., 1999). Antioxidants protect cells against damage caused by molecules known as free radicals. Oxidative damage is implicated in most disease processes such as
cardiovascular disease, cancer, inflammatory conditions, asthma, liver disease and macular degeneration. (Willcox et al.). Betalains are a class of nitrogen-containing pigments, the betalain pigment also interacts with their environment, such as attraction of pollinators and protection of tissue against damaging UV-radiation as well as increase pathogen resistance and improve viral defense especially for under ground growing part like red beet root.

Recently, natural plant pigments have been widely used as natural colorants to replace synthetic dyes. They are largely used as food colorants in food products like yogurts, ice cream and other products (Zhong et al., 2005) and (Stintzing et al., 2002). Nature has been a source of medicinal agents for thousands of years. Although advances have been made in pharmacology and synthetic organic chemistry, this reliance on natural products, particularly on plants, remains largely unchanged (Trevor et al., 2001).

DNA was extracted from leaf tissue using a modification of the cetyl trimethyl ammonium bromide (cTAB) method of Doyle and Doyle (1990). Polymerase chain reaction (PCR)-based techniques have been developed to evaluate DNA polymorphisms in a wide variety of organisms (Welsh & McClelland, 1990; Williams et. al., 1990). The rbcL gene encodes ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (Ellis, 1979). The rbcL gene has 700 bp in length and has conserve primer. The rbcL sequence can be used for cogeneric analysis (Kres et.al., 2005). The rbcL gene is an adaptive gene to environment heterogeneity and widely used for plant evolutionary and plant adaptation mechanism (Galmez et.al, 2005; Sem, 2011).
MATERIALS METHODS

PLANT COLLECTION

The plant *Amaranthus retroflexus* leaves was bought from the own land in anthiyur Erode (Dt). Good conditioned leaf sample was collected, due to morphological condition.

DNA EXTRACTION

The leaf sample was collected and it was extracted with the help of liquid nitrogen, the extract was made in to paste. And then add warm cTAB extraction buffer and mixed well. It was incubated at 10 to 60 minutes at 65°C. Add equal volume of isoamyl alcohol was added, mixed well by the inversion position, and centrifuged 8000rpm for 5 minutes at 4°C. The supernatant was collected, and it was added 1/10 volume of cTAB - NaCl solution and mixed well, then extract with equal volume of chloroform, isoamylalcohol. Centrifuged and collect the upper phase, 1volume of cTAB precipitation solution was added. Mixed well, centrifuged 1000rpm in 5 minutes at 4°C, and then precipitate was collected adding 0.6 volume of isopropanol mixed well and centrifuged for 8000rpm in 15 minutes at 4°C. The pellet was washed with 70% ethanol, dried, and resuspended in minimal volume of TE buffer.

PCR AMPLIFICATION

Polymerase chain reaction (PCR) of the isolated sample DNA barcode gene was carried out in a 20μl reaction mix containing 1X Taq buffer, Taq DNA polymerase, and genomic DNA. The PCR was performed using Agilent sure cycler 8800.

Agarose gel electrophoresis

Prepare agarose solution in the buffer provided (0.5xTEB) boil the agarose, until it is completely dissolved. When the gel temperature is around 40°C, add 2μl ethidium bromide and mix properly. Pour the agarose mixture in to tray and solidify. Then keep the gel tray containing 0.5x TEB buffer with the wells in the cathode (Negative) side. The buffer level in the tank should be maintained above the gel tray. Connect the cords between the electrophoresis tank and the power pack before loading the samples. To prepare samples for electrophoresis, add 5μl of gel loading dye in to the sample and mix well. Load 20μl of the sample and 3μl of DNA marker in to the well. After loading switch on the power pack and
adjust the voltage to 50V or 100V. Continue the electrophoresis until the dye reach the gel and observe the bands under UV trans illuminator.

**Sequence Information:**

The PCR products were purified and sequenced by Bangalour laboratory in QTlomics trainsts to genes.

**NCBI: Nucleotide Blast (BLASTn) output:**

The sequences obtained were subjected to BLAST using the BLAST N tool of NCBI to obtain the closest hit of the query sequence by Bangalor laboratory in QTlomics trainsts to genes.

**Phylogenetic Analysis: (Altschul et al., 1997)**

The rbcL gene sequences obtained from the *Amaranthus* strains were initially sequences available in the National Center for Biotechnology Information database using BLAST network services to determine their approximate phylogenetic affiliations. The sequences were aligned using Clustal W and the genetic distances were computed according to the Kimura 2-Parameter (K2P) model. The similarity values between the sequences were calculated from distance matrices by reversing the Jukes-Cantor distance formula. Phylogenetic trees were then inferred by neighbour joining (NJ) using the Kimura two-parameter model. The resulting NJ tree was evaluated by bootstrap analyses based. Finally, an overview of the phylogenetic position of *Amaranthus* was created by the rbcL gene sequences to corresponding sequences available in databases and the sequences obtained in this study for *Amaranthus retroflexus* evaluated with 1,000 bootstrap replicates.
RESULTS AND DISCUSSION

*Amaranthus* is a genus in the family of *Amaranthaceae*. The *Amaranthus* is multicomponent containing several different groups of subunits and polypeptides, thus it is characterized by essential molecular heterogeneity (Reema srivastava *et.al.*, 2012). Studies on the evolution relationships of the genus *Amaranthus* species cultivated and theirs wild relatives have been made especially in the last 20 years, by applying various techniques (Gabriela *et.al.*).

Morphological differences among *Amaranthus* variant can be analyzed using molecular marker, especially using DNA sequences. Phenotypic variation caused by plant morphological, functional, and developmental changing because of environmental heterogeneity to one or more genotype in one population (Radford, 1986). Medicinal plants contain biologically useful secondary compounds, including tannins, alkaloids, and polysaccharides, all of which can inhibit DNA extraction and amplification by co-precipitating with or binding to DNA (M. Schori and A.M. Showalter).

**PCR amplification**

Polymerase chain reaction (PCR) of the given sample with the candidate DNA barcode gene was carried out in a 20μl reaction mix containing 1X Taq buffer, Taq DNA polymerase, and genomic DNA. The PCR was performed using Agilent sure cycler 8800.

Most of the current methods to determine genetic diversity are based on polymerase chain reaction (PCR). Molecular methods have proved useful in the characterization of biological material.

The low level of polymorphism in these species may reflect the high level of inbreeding in these *Amaranthus* species or the fact that the primers which we have used they were not successful in amplifying bands from members of the genus *Amaranthus* (Bardini, *M.et.al.*, 2004).
Amplification check on gel

The PCR amplification profile was checked on agarose gel and the product size was compared with a size ladder of 100bp

![Image of gel](image.png)

**Figure1**: The PCR product was resolved on 2% Agarose gel at 120V for approximately 60 min or till the samples reached 3/4th of the gel. The gel was visualized under UV light and the image was captured. Lane 2: 100bp ladder, Lane 1: Amplification product of leaf sample with rbcL

**Sequence Information**:

The PCR products were purified and sent for Sanger sequencing. Below is the sequence information as obtained by Sanger sequencing.

If clean genomic DNA is obtained, appropriate primers are needed to amplify the targeted gene region. Certain gene regions, including rbcL have universal primers that work for most plants. There were used in this research, rbcL genes. The rbcL gene has 700 bp in length (Radford, 1986).
The sequences obtained were subjected to BLAST using the BLAST N tool of NCBI to obtain the closest hit of the query sequence.

(NCBI) Nucleotide Blast (BLASTn) output:

(A) rbcl
Figure 2: Showing NCBI-blast hit search result for the given leaf sample with rbcL gene specific primer. The blast search showed similarity to genus Amaranthus. The first 10 blast hit sequences were taken to construct a phylogenetic tree.

Phylogenetic Analysis:

The sequences were aligned using Clustal W, and the genetic distances were computed according to the Kimura 2-Parameter (K2P) model. Phylogenetic tree was constructed using UPGMA method on MEGA 6 software and the reliability of the tree was evaluated with 1,000 bootstrap replicates.
These studies have allowed the development of some hypotheses about the geographical origin of species and establish phylogenetic links between them. Also, there has been a series of tests on the molecular characterization of germ plasm in order to obtain useful results for breeders (Gabriela et al.).

(A) rbcL
CONCLUSION

In conclusion, this study showed that in spite of antigenic and allergenic differences among from Amaranthaceous family. This report will help us to find strategies for marker-assisted identification of amaranth genotypes in order to conservation of amaranth genetic resources or for different practical approaches. The results also suggest that species usually maintained high level of polymorphism over the cultivated counterpart. The high genetic variability caused by local adaptation and gene flow among species.

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REFERENCE

7. Gabriela , Calina Petruţa , Matilda , Narcisa , Ovidiu , Doru studies on genetic diversity in amaranthus species using the rapid markers.


15. M. Schori and A.M. Showalter DNA barcoding as a means for identifying Medicinal plants of Pakistan.


20. Trevor L (2001). Examining the potential role of co-operatives in the ethical commercialization of medicinal plants: Plant conservation, intellectual property, ethics,
and devils club (*Oplopanax horridus*), Occasional Paper Series Department of Biology University of Victoria.

