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GENETICS AND PLANT BREEDING

Screening and Identification of Genotypes for Natural Color from Grain Amaranth (*Amaranth* sp.)

Vasundhara M[1*], Sheetal R Gujaran[2], Marappa N[3], Niranjana Murthy[4], Lakshmi[5] and Priyanka, R[6]

[1,2,5,6] Department of Horticulture, UAS (B), GKVK, Bangalore, India
[3]Department of Genetics and Plant Breeding, College of Sericulture, Chintamani, India
[4] AICRN on Potential Crop Scheme, UAS(B), GKVK, Bangalore, India

*Corresponding author: vasundhara.vasu@gmail.com

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ABSTRACT

Nature exhibits a wide range of colours in the form of plant pigments that are used as dyes. Anthocyanin is one such water soluble pigment responsible for an attractive red to blue colour and is known to possess high antioxidant activity. Research on grain amaranth thus far has been focussed on production and productivity besides its nutritive quality. The present study highlights on extraction of anthocyanin from grain amaranth, an underutilized crop. Total anthocyanin content (TAC) (mg/g) in leaf, inflorescence and stem peel was estimated and evaluated statistically (1% and 5% level of significance). Leaves, stem-peel and inflorescence of grain amaranth considered as a waste material after seed extraction is apotential source of anthocyanin adds value.

Highlights

- There is wide variability and is a waste product after harvesting the seeds a natural source of anthocyanin pigment can be used for eco-friendly products

Keywords: Anthocyanin,Grain Amaranth, Inflorescence, Leaves

Underutilized food crops indigenous to the third world, but neglected by researchers and policy makers are now being considered. Grain Amaranth (family: Amaranthaceae) is one of the many such little known, underexploited plant with an exceptional nutritive value (Singhal and Kulkarni 1988). It is a fast growing, dicotyledonous belonging to genus Amaranthus. The genus has more than 60 species of which some are grown for leaf purpose, seeds of which are black in color. Three species are grown for grain purpose, seeds of which are golden yellow in color. They are very nutritious and popularly called as 'Dantina Beeja' in kannada or 'Rajgira' in Hindi. Amaranth grain, rediscovered in modern agriculture, has remarkable nutritive quality due to the amount and quality of protein as lysine content (14-16%) an essential amino acid. The grain is also rich in calcium, phosphorous and iron. The crop can be raised in all the three seasons of the year, *Khariff* (May-June), *Rabi* (Oct-Nov) and in Summer (Jan-Feb). Amaranth yields about 15-20 quintal/hectare of seeds under irrigation, with a biomass of 8-10 quintal/hectare, which goes as a waste. There is a wide variability in anthocyanin pigment was observed in Amaranth collections of All India Co-ordinated Research Network on potential crops at UAS (B), Bangalore.

Also, another, interesting application of amaranth is that, people with allergies to other grains prefer it (Kauffman and Weber 1990). Grain Amaranth is a fast growing, high yielding, stress resistant and requires little cultivation inputs. It is rich in protein, fat, dietary fibre and minerals especially calcium, iron, potassium, sodium and has a superior amino acid profile compared to other plant foods (Muyonga *et al.* 2014). The antioxidant activity of grain amaranth is attributed to polyphenols, flavonoids and tocopherols (Klimczak *et al.* 2002; Pasko *et al.* 2009). All these nutritional benefits collectively offer opportunities to utilize this grain as a therapeutic adjunct particularly in countries with nutritionally deficient diets. It is one of the few multi-purpose crop which can supply grains and tasty leafy vegetables of high nutritional quality as a food and animal feed, and additionally, because of attractive inflorescence coloration, amaranth can be cultivated as an ornamental plant.

There has always been an increased interest in tapping out various natural sources of food colourants. The need arises due to the side effects posed due to the usage of synthetic food colourants. The role of water soluble pigments, anthocyanins, become important as they are responsible for the reds and blues of many fruits and vegetables (Fossen *et al.* 1998). In addition, anthocyanins are well known antioxidants known for their anti-inflammatory and analgesic effects. (Diaconeasa *et al.* 2015). Due to its possible health benefits there is an increasing interest in estimating the anthocyanin content in food and nutraceuticals. Anthocyanin pigment can also be used in quality control and purchase specifications of fruit juices, nutraceuticals and natural colourants. (Lee 2005). The potential sources of anthocyanin include grapes, red cabbage, elder berry, black carrot, black currant (Fossen *et al.* 1998). There is a huge variability of anthocyanins spread in nature. The glycoside derivatives of the three non-methylated anthocyanidins (Cyanidins, Pelargonidin and Delphinidin) are the most common in nature being found in 80% pigmented leaves, 69% in fruits and 50% in flowers (Ovando *et al.* 2009).

The work carried out till date has been concentrated on increasing seed yield, oil, and nutritive quality of grain amaranth while, the information available on the anthocyanin pigment is nil. There is a need to fully utilize the underutilized crop for its pigment as there is a wide variability and becomes a waste after extracting the seeds. Our study was aimed at estimating anthocyanin content in the leaves, inflorescence and stem -peel of nine grain amaranth accessions.

MATERIALS AND METHODS

1. Collection and Processing of Plant Material

Accessions (1 to 9) of Grain Amaranth was procured from All India Coordinated Research Network on Under Utilized Crops, GKVK, UAS(B), Bangalore, India.

2. Sample Preparation

The leaf, inflorescence and stem peel were separated and dried at room temperature for 3-4 days. The dried plant material was powdered using liquid nitrogen and stored at 8ºC for further analysis (Fig. 1).

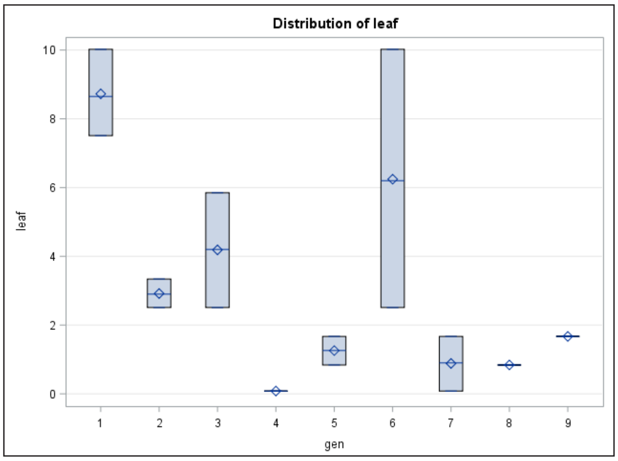

Fig. 1: Variation of mean TAC (mg/g) in leaf among the accesions of Grain Amaranth. (Note : Accesions are labelled from 1 to 9)

3. Extraction of Anthocyanins

Anthocyanins were extracted from powdered leaves, stem-peel and inflorescence. The extraction was carried out as per the protocol in Liu *et al.* 2012 with slight modifications. Briefly, the freeze-dried powder (0.5g) were added to 7.85ml 60% ethanol containing 0.1%(v/v) hydrochloric acid and placed in hot water bath for 116.88 min at 64.38ºC. The samples were centrifuged at 2700 rpm for 10min @ 25ºC.

The supernatant was filtered through 0.45µm syringe filter. The undiluted final filtrate was used to quantify anthocyanins present in the samples (Fig. 2 and Fig. 3).

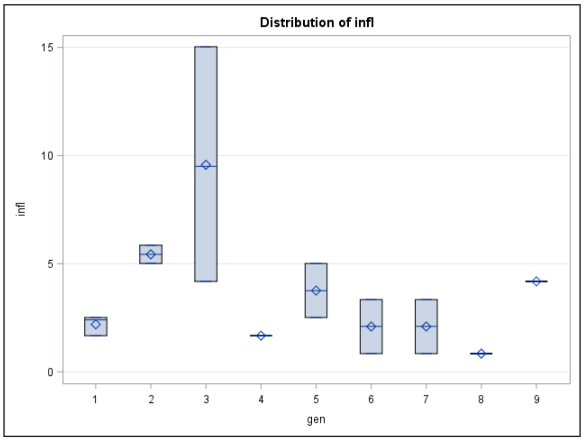

Fig. 2: Variation of mean TAC (mg/g) in inflorescence among the accesions of Grain Amaranth. (Note : Accesions are labelled from 1 to 9)

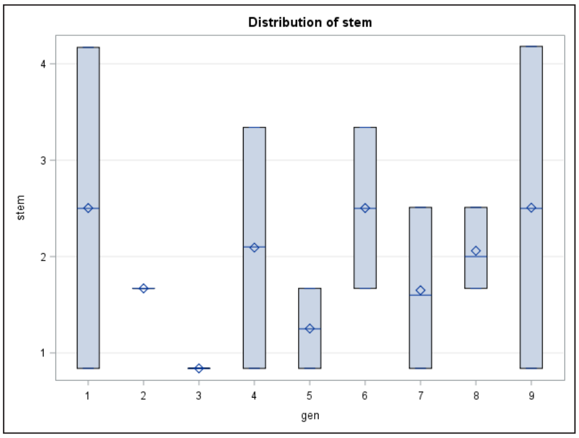

Fig. 3: Variation of mean TAC (mg/g) in stem among the accesions of Grain Amaranth. (Note : Accesions are labelled from 1 to 9)

4. Total Anthocyanin Content Measurement using pH Differential method

The total anthocyanin content was determined according to spectrophotometric pH-differential method (Liu *et al.* 2012). Briefly, an aliquot (1ml) of the extract was mixed with 0.025M potassium chloride buffer (pH 1.0, 4ml) and 0.4 M sodium acetate buffer (pH 4.5, 4ml) respectively. The absorbance of the mixture was measured at 510nm and 700nm using UV-V is spectrophotometer. Absorbance was calculated as $A = [(A_{510} - A_{700})$ at pH 1.0] - $[(A_{510} - A_{700})$ at pH 4.5] with a molecular extinction coefficient of 26,900 for anthocyanin. The total anthocyanin was calculated as cyaniding-3-glucoside equivalents as per the following equation:

$$\text{Anthocyanin, (mg/g)} = (A \times MW \times DF \times V \times 103)/(\varepsilon \times L \times m)$$

A- Absorbance; MW- Molecular weight of cyanidin-3-glucoside (449.2 Da); DF- Dilution factor; V is the final volume (ml); L- cell path length(1cm); m- weight of the dried powder taken (g).

5. Statistical Analysis

All the experiments were carried out in duplicates and the results were expressed as Mean±SE mg/g. Statistical analysis was performed using SAS system. Value of $p<0.05$ and $p<0.01$ was considered statistically significant.

RESULTS AND DISCUSSION

In the present study, the extraction method of anthocyanin from different parts of amaranth, viz., leaf, inflorescence and stem-peel was evaluated spectrophotometrically by AOAC pH differential method. This method is extensively been used by food technologists and agriculturists to assess the quality of fresh and processed fruits and vegetables (Lee 2005, Sai Srinivas 2017). In the present study, the Total Anthocyanin Content (TAC) (mg/g) extracted from leaf and inflorescence of grain amaranth displayed significance, while, the TAC of stem peel was non-significant (Table 1).

Table 1: Total Anthocyanin Content (TAC) (mg/g) in leaves, Inflorescence and Stem-peel of Grain Amaranth

Sl. No.	Accession	TAC (mg/g)		
		Leaves	Inflorescence	Stem peel
1	SKGPA-72	8.73±0.726	2.19±0.264	2.50±0.961
2	KBGA-1	2.92±0.240	5.43±0.242	1.67±0.000
3	GA-2	4.18±0.964	9.57±3.132	0.84±0.000
4	KBGA-3	0.08±0.001	1.67±0.000	2.09±0.722
5	SKGPA908	1.26±0.240	3.76±0.722	1.25±0.240
6	ICO3573	6.24±2.168	2.09±0.722	2.50±0.482
7	IC033701	0.88±0.458	2.09±0.722	1.65±0.483
8	KBGA-4	0.84±0.000	0.84±0.000	2.06±0.244
9	BGA-11	1.67±0.00	4.18±0.000	2.51±0.964
Accession MSS		25.2019**	21.4408**	1.0560-NS
Replication MSS		0.2942	0.0791	3.265

Values are expressed in Mean ± SEM, mean of 3 independent replications.

The TAC of leaves was in the range of 0.080-8.727 mg/g. Out of 9 accessions being evaluated, accession SKGPA-72 recorded higher TAC of 8.727mg/g followed by accession ICO3573

(6.243mg/g) and accession GA-2 (4.187 mg/g) (Fig. 4). In inflorescences, TAC of 0.840- 9.570 mg/g was recorded, wherein, GA-2 had the highest mean TAC value (mg/g) of 9.570 mg/g. While, accession KBGA-1 (5.430mg/g) and accession SKGPA-908 recorded 3.757mg/g (Fig. 5). In Stem-peel, TAC was between (0.84-2.5067mg/g) andit was observed that the TAC value ofaccessions SKGPA-72, ICO3573 and BGA-11 were on par with each other (2.507mg/g) (Fig. 6). On comparison, the mean TAC value in inflorescence was greater followed by leaves and Stem-peel.

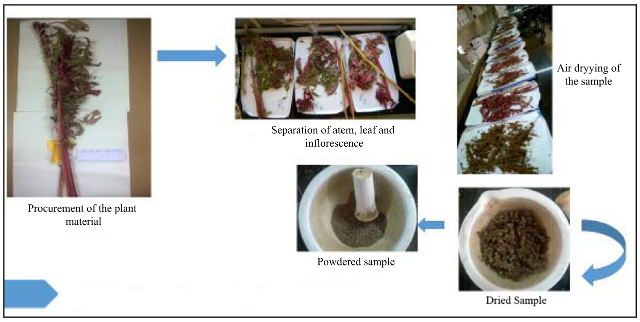

Fig. 4: Collection and Processing: Amarnath Plant Material

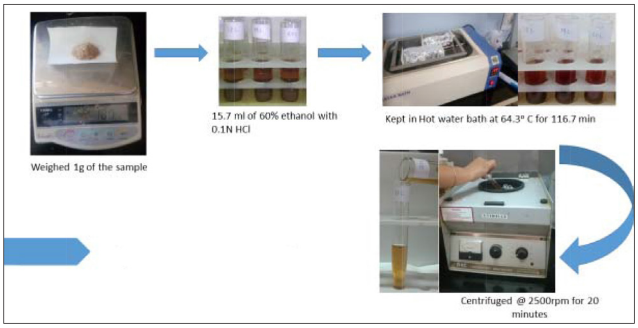

Fig. 5: Extraction of Anthocyanin

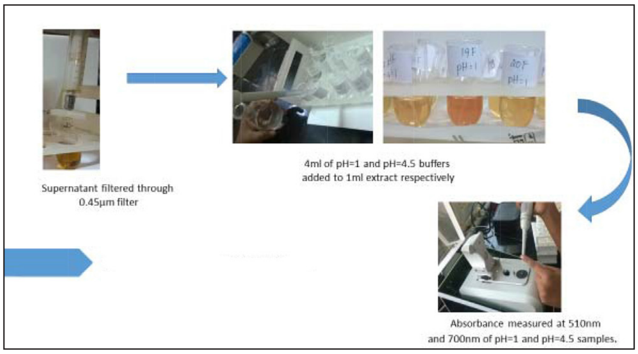

Fig. 6: Quantification of Anthocyanin

A considerable variation in the total anthocyanin content (TAC) and the nature of the pigment is observed in fruits and vegetables. Anthocyanins in fruits vary from 0.006-3.90mg/g, in vegetables 0.022-3.22 mg/g, in beverages (Wine and grape juice) 0.107-0.14 mg/g and about 0.075 mg/g in pistachio nut (Wu *et al.* 2006). *Amaranth cruentus* is reported to contain an anthocyanin content varied from 0.908-0.104mg/g (Pasko *et al.* 2009).

Anthocyanin pigments play an important function in plant physiology as they play a major role in pollination, seed dispersal and also protect the leaf from UV light (Wu *et al.* 2006). The role of anthocyanin pigments as medicinal agents have been well-accepted dogma in folk medicine throughout the world and have multifaceted roles in human health maintenance. These pigments and associated flavonoids have demonstrated ability to protect against a myriad of human diseases. They improve the visual acuity by enhancement of rhodopsin regeneration. Recent studies have confirmed their potential in the protection against liver injuries, reduction in blood pressure, strong anti-inflammatory activity, antimicrobial, antioxidant activity and suppression of proliferative human cancer cells, reduce the risk of cardiovascular diseases, and also against age related neurodegenerative diseases (Lila 2004; Konczak and Zhang 2004; Han and Xu 2014). Anthocyanin pigments and other flavonoids that are produced in grain amaranth can be of great advantage for health and nutrition research because they are quickly and easily isolated and are also concentrated in all parts. In comparison with the total anthocyanin content of fruits, vegetables and juices; leaves, inflorescence and stem peel of grain amaranth in the present study recorded a higher anthocyanin pigment and thus can also be utilized as a potential source of this natural pigment. Additionally, the total biomass of grain amaranth varies from 720-1320g/m^2 of which, 140-300g/m^2 constitutes grain yield. In other words, of the total biomass, ~20% of the biomass corresponds to the grain yield, while the remaining 80% is considered as an agricultural waste. The present study thus highlights the utilization of this biomass from an underexploited crop as an economical source of natural pigment besides its nutritive grains and also as an economical way to manage waste.

CONCLUSION

Grain Amaranth, a nutrient rich and an underutilized cereal can play an important role in actions against malnutrition. This grain is very versatile as a food ingredient and can diversify farming enterprise, as it can be used to prevent food depletion and to feed the world. The use of alternative crops would result in product competitiveness and diet diversity and thus boost productivity. There is a need to fully utilize the underutilized crop for its pigment as there is a wide variability and is a waste product after harvesting the seeds. Therefore, these results show that apart from the seeds, all other parts of Grain Amaranth usually treated as a waste can be utilized as a natural source of anthocyanin pigment and can be proposed to be part of the production of eco-friendly products.

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