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Research Journal of Agricultural Sciences
Volume 14; Issue 02 (Mar-Apr 2023); pp 552–558

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Research Review

Taxonomic Delimitation and Biotechnological Management of Nutrient and Antinutrient Factors in Vegetable Amaranths - A Review

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Abstract

In hot humid tropics of Africa, South-East Asia, vegetable Amaranths are recognized as the most favorite leafy vegetable for their mild spinach-like taste and immense nutritive value. Along with a range of nutrients like – proteins, minerals, vitamins, dietary fibers, antioxidants, bioactive principles, they also contain few antinutrients like oxalate and nitrate. Seeds of grain Amaranths have been identified as the richest plant source of Squalene, a natural antioxidant but vegetable Amaranths are yet to be evaluated for Squalene. Presence of a large number of crop-wild relatives, morphotypes, landraces, having almost equal palatability have disputed the taxonomic delimitation in vegetable Amaranths. Taxonomic ambiguity in vegetable Amaranths has been resolved, enabling easy identification of crop wild relatives from the cultivated popular members. Crop improvement of vegetable Amaranths require 2-fold approaches – firstly, screening of all the existing germplasms to identify the suitable ones rich in nutrient and antioxidants for large scale utilization as vegetable, Secondly, application of Biotechnological strategies to improve the amount and availability of nutrients in crop as well as decrease the level of antinutrients. *Agrobacterium* - mediated transformation protocol standardized in *Amaranthus tricolor* has consolidated the feasibility for crop improvement applying Biotechnological strategies producing transgenic plant with many novel genes.

***Key words*:** Vegetable amaranths, Nutrients, Antinutrients, Biotechnology, *Agrobacterium*–mediated transformation

Amaranthus is an herbaceous cosmopolitan genus of Amaranthaceae, collectively called pigweed or Amaranths. According to Iamonico [1], it includes approximately 70 species, 40 of which are suggested to be the native of America. Sauer [2] recorded 75 species under *Amaranthus*, mentioning availability of 25 species in Asian region. The fossil pollens recovered from the sediments that date back to the Holocene and late Palaeocene period evidence the antiquity of *Amaranthus* in Indian sub-continent. They are generally annual weeds, few species are popular as vegetables and protein enriched rich seeds of few species (*A. hypochondriacus*, *A. cruentus* and *A. caudatus*) are utilized as pseudo-cereals, commonly known as grain amaranths.

Amaranths are recognized as one of few multipurpose crops. Taxonomic delimitation in Amaranths, especially in vegetable Amaranths are ambiguous due to significant morphological variability, absence of adequate species defining characteristics, introgression and hybridization between conventional cultivars and weedy species [3] and presence of a large number of crop-wild relatives, landraces and morphotypes. Scattered attempts towards classification, nomenclature, and geographical distribution were made by few workers [4-5]. Vegetable amaranths are the most popular vegetable crops grown in the hot and humid tropical low-lands of Africa and Asia for their protein-rich leaves and stems [6]. They are very rich in carbohydrate, lysine-rich protein, minerals specially Calcium, Vitamin A, K, C, Folate, Riboflavin, B6, dietary fiber, an array of antioxidants and bioactive components. High nutritive value, mild spinach like flavor, least agricultural requirements and high yield and ability to grow in hot weather, are few plus points for their popularity. Leaves of most *Amaranthus* species are edible but few of them are consumed as vegetable like – *A. tricolor*, *A. blitum*, *A. dubius*, *A. cruentus* and *A. viridis*, the first two are most popular in hot humid tropics of Asia and South-East Asia. Ethnobotanical survey confirms the presence of many land races and crop-wild relatives of both *A. tricolor* and *A. blitum* which can perform the role of nutrient rich palatable minor crops. In comparison with major crop this underutilized and neglected crop-wild relatives require low input, not competitive to the conventional crop and have the potentiality to become economically viable.

Received: 12 Dec 2022; Revised accepted: 24 Mar 2023; Published online: 21 Apr 2023

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Citation: Das S. 2023. Taxonomic delimitation and biotechnological management of nutrient and antinutrient factors in vegetable Amaranths – A Review. *Res. Jr. Agril. Sci*. 14(2): 552-558.

Large scale utilization and attempts towards broadening the list of vegetables are somewhat hindered by few factors, like - disputes in taxonomic delimitation of useful germplasms of vegetable Amaranths belonging crop-wild relatives and morphotypes, presence of few antinutrient components and absence of proper screening of all existing germplasms in terms of nutrient and antinutrient elements. Recent advances in biotechnological methodologies have boosted the feasibility of crop improvement by managing the level of nutrients and antinutrients. The amount and availability of nutrients now can be improved applying strategies like, simple plant selection for nutrient-rich high-yielding varieties, cross breeding to incorporate desired trait and genetic engineering to manipulate the nutrient, antinutrient content followed by multilocation trials to select types with wide adaptability. Recent advances in genetic manipulation and standardization of *Agrobacterium*-mediated transformation in vegetable Amaranthus have brightened the feasibility of crop improvement in desired direction.

Fig 1 Common colourmorphs of popular leafy vegetable *A. tricolor* var. *tricolor*

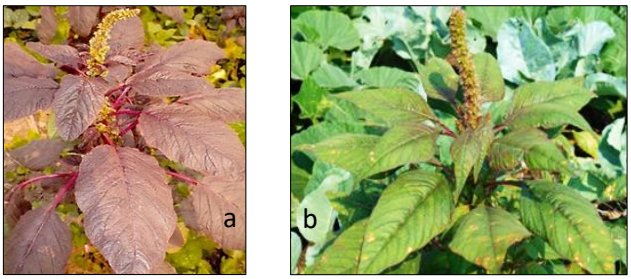

Fig 2 Crop-wild relatives of Tricolor complex – (a) *A. tricolor* var. *acutus* and (b) *A. parganensis*

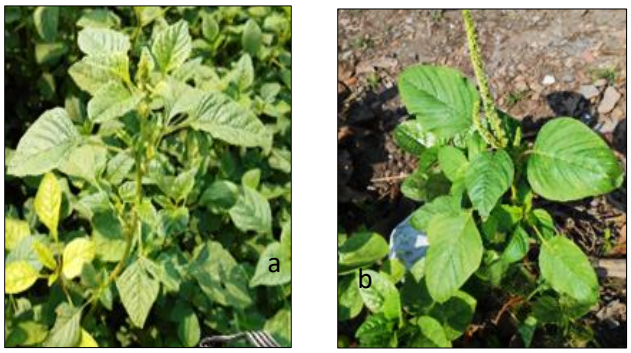

Fig 3 Blitum complex with – (a) popular member *A. bengalense* and (b) crop-wild relative *A. blitum* var. *oleraceus*

Morphological and taxonomic variability in vegetable amaranths

Leaves of most *Amaranthus* species are edible, but few are very popular as vegetable, e.g., *A. tricolor* L., *A. blitum* L., *A. dubius* L., *A. cruentus* L., *A. viridis* L. etc., of which *A. tricolor* and *A. blitum* are two most popular vegetables in humid tropic. Both *A. tricolor* and *A. blitum* are enriched with many land races, morphotypes and crop-wild relatives. Two species complex or aggregates has been established to resolve the taxonomic dispute, namely - Tricolor complex [7-9] and Blitum complex. After comprehensive field survey the configuration of Tricolor complex was simplified, straight away including three varieties namely – *Amaranths tricolor* var. *tricolor* L., *A. tricolor* var. *tristis* L. and *A. tricolor* L. var. *acutus* S. Das. [9] ignoring the synonyms. *Amaranthus tricolor* var. *tricolor* is enriched with a number of colourmorphs (Fig 1). A gynomonoecious crop-wild relative of *A. tricolor*, *Amaranthus parganensis* Saubhik Das, was identified from lower gangetic plain of West Bengal [10] and was included in the Tricolor complex. Both *A. tricolor* var. *acutus* and *A. parganensis* are less known crop wild relatives in Tricolor complex (Fig 2). Blitum complex includes four taxa – *A. blitum* L., *A. blitum* var. *oleraceus* (L.) Hook. f., *A. emerginatus* Salzm. Ex Uline & Bray, *A. emerginatus* var. *pseudogracilis* (Thell.) [11]. In Indian subcontinent specially in the Gangetic plain of West Bengal, Blitum complex is represented by two varieties of *A. Blitum* – *A. Blitum* var. *blitum* L. and *A. blitum* var. *oleraceus* (L.) Hook. f. and *A. bengalense* Saubhik Das & Iamonico [12], where *A. blitum* var. *oleraceus* is a neglected crop-wild relative (Fig 3) of Blitum complex. The crop-wild relatives or neglected landraces of both the species complexes are underutilized in spite of having almost identical taste and palatability like conventional popular crop and deserve to be evaluated nutritionally.

Nutritive value of vegetable amaranths

Vegetable amaranths are rich source of minerals like - iron, magnesium, potassium, phosphorus, lysine-rich protein, riboflavin, niacin, vitamin B_6, A, C, E, and K, folate, dietary fiber, phytosterols and useful antioxidants. Investigations by Prakash and Pal [13] have revealed a high nutritive value of vegetable amaranths, even much better than popular spinach and cabbage (Table 1). Amaranth is ranked as one of the top five vegetables in antioxidant content, enriched with a range of bioactive elements like - β-carotene, L-ascorbic acid, polyphenol, anthocyanins and lutein [14]. In comparison with other plant sources, namely, rice, wheat, rye and oats vegetable Amaranths contains 30% more gluten free protein with complete set of amino acids [15].

In the biosynthetic pathway of Cholesterol, Squalene is an intermediate triterpene with great implications in food, cosmetic, and pharmaceutical industry. Its potential application in cosmetic dermatology, biological and pharmacological roles as well, were comprehensively reviewed [16]. Squalene is reported to be present in all vegetable oils though in small amounts. Shark liver oil is considered as the richest and known source of Squalene, however, significant amounts are also found in wheat-germ, olive, Amaranth grain, palm, rice bran oils, vegetables and microorganisms. The squalene content of olive, wheat germ, and rice bran oils ranges from 0.1 to 0.7% [17]. The overall oil content in Amaranth grain varies from 1.9% to 8.7% with an average of 5.0% and average squalene concentration in extracted oil measured 4.2% with a range from trace to 7.3%. Investigation on dried mature leaves of 45 *Amaranthus* genotypes showed a range of fat content from 1.08 to 2.18% with an average of 1.63% but the squalene concentration appeared much lower than from seeds, averaged 0.26% with a range from trace to 0.77% [18]. A methodology for isolation and purification of squalene from *Amaranthus* oil was developed by He *et al*. [17]. Squalene is a natural antioxidant, prevents H_2O_2 induced oxidative injury and protect against oxidative DNA damage [19], reduces serum cholesterol [20], participates in the formation of steroid hormones, bile acids, steroids, and sterols [21].

Table 1 Comparative nutrient profile of vegetable amaranth, spinach and cabbage

Components	Cabbage raw (value/100g)	Chinese cabbage raw (Value /100 g)	Spinach raw (Value / 100g)	Amaranth raw (Value 100 g)	Amaranth cooked (Value /100 g)
Proteins (g)	1.28	1.20	2.86	2.46	2.11
		Minerals			
Calcium (mg)	40	77	99	215	209
Iron (mg)	0.47	0.31	2.71	2.32	2.26
Magnesium (mg)	12.0	12.0	79.0	55.0	55.0
Phosphorus (mg)	26.0	29.0	49.0	50.0	72.0
Potassium (mg)	170.0	238.0	558.0	611.0	641.0
Sodium (mg)	18.0	9.0	79.0	20.0	21.0
Zinc (mg)	0.18	0.23	0.53	0.90	0.88
Copper (mg)	0.019	0.036	0.136	0.162	0.158
Manganese (mg)	0.160	0.190	0.897	0.885	0.861
		Vitamins			
Vitamin C (mg)	36.6	27.0	28.1	43.3	41.1
Riboflavin (mg)	0.040	0.050	0.189	0.158	0.134
Niacin (mg)	0.234	0.4	0.724	0.658	0.559
Vitamin B6	0.124	0.232	0.195	0.192	0.177
Folic acid (mcg)	43.0	79.0	194.0	85.0	57.0
Vitamin A RAE 1 (mcg)	5.0	16.0	469.0	146.0	139.0
Vitamin K	76.0	42.9	482.9	1140.0	-
		Lipids			
Total saturated fatty acids (g)	0.034	0.043	0.063	0.091	0.050

Source: USDA National Nutrient Database or standard reference, Release 23 (2010)

The preparation of leaf protein concentrate is a promising approach in nutrition biology. Leaf protein of vegetable Amaranths are highly extractable in comparison with other species. Along with leaf protein, several other valuable nutrients like Provitamin A (β – Carotene), Polyunsaturated lipids and Iron are also extracted. Treatment of the extract with acid precipitates the nutrients as leaf-protein concentrate while most of the harmful compounds remains in the soup and eliminated. The cheese-like green coagulum is then washed with water, acidified with vinegar to minimize the level of antinutrients. This leaf-nutrient concentrate is very useful specially for children for its digestibility and excellent nutritive effectiveness [22-23].

Antinutrient factors

The chemical components which are undesirable and abundant in both cultivated and wild plant species, also known to reduce the bioavailability of nutrients in the body, is referred to as anti-nutrients [24]. Oxalates and nitrates are two common anti-nutrient factors found in leafy vegetables. Oxalic acid is available in the cell sap of all most all green leafy vegetables may reduce the availability of certain minerals in the body particularly calcium, when present in excess amount. An intake of vegetables with high dietary oxalate may inhibit or influence the absorption of minerals and trace elements in humans and may lead to formation of calcium oxalate crystals that can be stored in the kidney causing Kidney stone.

Generally, the leaves contain high amounts of oxalates followed by seeds, while stems contain the least [25]. The amount of oxalate varies from plant to plant also from species to species. The leaves of *Chenopodium album* (goosefoot) contain oxalate ranging between 394.19 mg/100g to 518.42 mg/100g [26] while leaves of *Amaranthus* have Oxalate in the range of 0.94 % to 1.29% [27]. Though investigation has shown that consumption of 200 gm of cooked Amaranth per day does not create any health problem.

Nitrate content in leafy vegetable is also of concern because it is speculated that Nitrates may be transformed chemically into poisonous and carcinogenic nitrosamines in the digestive tract, though it is yet to be supported by any evidence. The leafy vegetables are reported to contain higher amounts of nitrates in comparison with root and fruit vegetables [28]. The World Health Organization has set the acceptable daily intake of nitrate at 3.7 mg/kg body weight and nitrite at 0.06 mg/kg body weight [29]. Nitrate content of leaves in *Amaranthus* varies from 0.55% to 1.0% [27]. Various agronomic practices such as the amount, timing and form of Nitrogen fertilizer applied as well as environmental and genetic factors can significantly influence the levels of nitrate in raw green leafy vegetables [30]. However, after inactivation or removal of such antinutrients by adopting economically and nutritionally viable indigenous processing techniques, the leafy vegetable can serve as more nutritive food source.

Screening of vegetable amaranths for nutrient and antinutrient factors

In most of the developing countries where the daily diet is dominated by starchy staple foods, vegetables can form the cheapest and most reliable, readily available sources of important vitamins, minerals, fibres, essential amino acids and antioxidants. A number of studies on nutrient content have emphasized the need of screening of all the existing germplasm of vegetable Amaranths to evaluate them in terms of nutrient, antinutrient and antioxidant content, also to enable the selection of suitable ones for large scale utilization. The nutrient components of the conventional vegetables are well documented but those of the crop-wild relatives, land races and less known morphotypes are yet to be evaluated properly. Squalene content has been mainly estimated in the grains of *Amaranthus*, but juicy stems and leaves of palatable vegetable Amaranths are quite ignored though *Amaranthus* has been proved to be an ideal source of Squalene.

The vegetable amaranths are semi-cultivated as rotational crop or collected from wild, harvested by uprooting or clipping at regular interval. Their cultivation practice is very simple and optimal conditions to maximize yield are also not fixed due to their wide adaptability. Investigations on vegetable Amaranths have been more emphasized than the grain

Amaranths in Asia, more specially in South-east Asia. Several important aspects are still to be looked into for further improvement–

1. Leaf yield and leaf/stem ratio
2. Content of Nutrients and nutrient uptake at different phases of crop growth and harvest.
3. Delayed appearance of Inflorescence
4. Proper timing of harvest and regrowth.
5. Crop quality, including tenderness and innovative adequate storage methods to prolong the life of the harvested material.
6. Accumulation of heavy metals and anti-nutrient factors in response to quality and quantity fertilizers applied and soil types.

The high yielding cultivar lines of conventional crops, landraces or their crop-wild relatives, need to be screened through multilocation trials to select types with wide adaptability. This will also be helpful to identify geographical locations where a given type will grow vigorously. Studies on genetic divergence, inheritance pattern and combining ability are also prerequisites for varietal improvement. Stability of landraces or crop-wild relatives against adverse condition, high genetic variability and disease resistance properties signifies their importance in plant breeding program. Vegetable Amaranth has a rich stock of landraces with a great number homozygote genotypes [31] enriching useable genetic variation.

Biotechnological approaches to develop genetically modified vegetable with desired outcome

Improvement of nutritive value by agrobacterium mediated transformation

Technological innovation in plant biotechnology is an important catalyst in augmenting any crop improvement program. Biotechnological strategies are now available to improve the amount as well as availability of useful nutrients in crop. Efforts have been made to improve protein quality and quantity in crop plants but with limited success. Biotechnological approaches have been applied successfully in grain *Amaranthus*, a novel protein gene *AmA1* has been isolated from the seeds of *Amaranthus hypochondriacus* and subsequently purified and characterized, its cDNA cloned [32]. The *AmA 1* protein is nonallergic, rich in all essential amino acid composition that correspond well with the standard optimized by World Health Organization for human nutrition. Transgenic potato plant has been raised through introduction of *AmA1* protein gene by *Agrobacterium* – mediated transformation, that has opened up a new avenue to improve the nutritive value of several other crop plants. In potato plant, the *AmA1* coding sequence was successfully expressed in tuber specific and constitutive manner, resulted an increase in total protein content (up to 60%) with concomitant increase in most essential amino acid components [33]. The qualitative and quantitative improvement of protein in vegetable Amaranths can also be achieved through transgenesis with *AmA1* gene, but that require a stable *Agrobacterium* – mediated genetic transformation protocol. The gene can be transferred to the whole plant by adopting either tissue infiltration technique [34] or floral dip method [35].

A protocol for *Agrobacterium* – mediated genetic transformation of *A. tricolor* was standardized through cocultivation of explant with *Agrobacterium rhizogenes* [36]. A genetic transformation protocol for majority of leafy vegetable crop was optimized [37] co-cultivating epicotylar explant of *Amaranthus tricolo*r L. with *Agrobacterium tumefaciens*. Several key factors influencing and regulating the transformation events were standardized. An invitro culture system was developed for utilization in plant regeneration and genetic transformation protocol [38] using the mature embryos of *Amaranthus hypochondriacus*. *Agrobacterium* – mediated transformation through floral dip has been evolved and successfully applied in the popular model plant such as *Arabidopsis thaliana* and *Medicago truncatula*. In this process transformation through floral dip followed by rapid selection of transformants has become phenomenal approach as it will help to overcome the probable difficulties during lengthy tissue culture procedure as well as screening transformed progenies [39]. The suspension of bacterial cells (*Agrobacterium tumefaciens* strain, AGL 1) having constructed vectors with selectable marker gene for Hygromycin was applied to *Amaranthus* inflorescence drop by drop and inflorescence left to produce seed. The seedlings raised from T_1 seeds were screened through Hygromycin spray. All the positive putative transformants also showed positive result when hygromycin applied on leaf disc. *Amaranthus* inflorescences could be transformed easily and the transformed progenies could be identified through a combination of simple and rapid methods. This encouraging result have rejuvenated the ideas of improving the crop and the crop wild relatives applying biotechnological methods. Conventional breeding methods relies on mixing features from different populations within a species followed by selection. However genetic engineering ensures inserting genetic element in random location that can destabilize complex gene interaction.

Transformation experiment using different plant parts of various *Amaranthus* species have been carried out by many workers, like transformation of *A. retroflexus* [40], *A, cruentus* [41-42] using inflorescence applying floral-dip method. Transgenic *A. caudatus* and transgenesis of hybrid plant between *A. caudatus* and *A. paniculatus* were achieved applying floral dip method [43-44]. Transgenic callus for *A. tristis* was obtained through transformation of leafy part with *Agrobacterium tumefaciens* strain having p^{CAMBIA} [45]. Plant regeneration have been achieved in nine *Amaranthus* species (e.g., *A. tricolor*, *A. paniculatus*, *A. spinosus*, *A. gangeticus*), and transformation for six species (e.g., *A. tricolor*, *A. viridis*, *A. retroflexus*) while tissue and organ transformations were achieved in four species (e.g., *A. tristis*, *A. tricolor*, *A. caudatus*) [46]. A protocol was optimized to transform roots of three grain Amaranths and *A. hybridus*, their putative progenitor by *Agrobacterium rhizogenes* - mediated transformation [47].

Improvement of nutritive value through accumulation of squalene

Amaranth seeds are reported to be a rich source squalene, beside other valuable nutrients. A large number of vegetable amaranths including unexplored crop-wild relatives and weeds needs to be assessed for commercial utilization as a potent source of Squalene. Few reports are available on methods of extracting and purifying squalene from *Amaranthus* seeds [17-18] [48]. In order to understand the regulation of Squalene biosynthesis in *Amaranthus*, a Squalene Synthase (*SQS*) gene was isolated from *Amaranthus* and characterized. Squalene Synthase (*SQS*) gene was isolated from *Amaranthus cruentus*, its' cDNA cloned and characterized as well as its' expression pattern was studied in seed at different developmental stages and in several tissues as well [49]. The full-length 1805-bp long cDNA encoding a protein comprising 416 amino acids with a molecular mass of 47.6 kDa was characterized. The envisaged

amino acid sequence of the *SQS* cDNA shared striking homology with those from several other plants. The enzyme Squalene synthase catalyses a 2-step reaction, firstly two Farnesyl diphosphate molecules get condensed to form an intermediate Presqualene diphosphate, which later transforms into Squalene by reductive rearrangement [50]. Gene encoding Squalene synthase has been isolated from many sources, such as fungi [51], bacteria [52], animals [53] and plants [54-56]. In some organisms there is only one copy of the *SQS* gene in the genome, while other organisms have multiple copies in their genome. A significant level of *SQS* transcripts was detected in the roots of Amaranths and other plants. The highest level of transcripts was detected in the stems, followed by leaves and petioles [49]. The expression levels of *SQS* gene in stem and root tissues are significantly higher than those in leaf tissues. Conventional vegetable Amaranths along with their crop-wild relatives need to be assessed for Squalene level in root, stem and leaves also to detect the copy number of *SQS* gene in the genome.

Biotechnological approach in lowering the level of Antinutrient

Oxalic acid is a common antinutrient factor present in almost all leafy vegetable in varying amount. Since green leaves are one of the main sources of other useful nutrients like – essential amino acid-rich proteins, minerals, β- Carotene and dietary fibers, their consumption cannot be ignored or discouraged. Two approaches are now visible on card, screening of all the germplasms of vegetable Amaranths including less known crop-wild relatives for antinutrient content and improvement of existing popular germplasms by lowering the oxalic acid content. In both the cases biotechnological approach has appeared adequate and effective. Different metabolic pathways of oxalate biosynthesis have been explored to evaluate the feasibility of reducing Oxalate levels in some crop plants [57]. Several pathways for Oxalate biosynthesis were hypothesized, including photorespiratory glycolate/glyoxylate oxidation, cleavage of ascorbate, hydrolysis of oxaloacetate [58-60].

Oxalic acid is degraded by two major pathways i.e., decarboxylation and oxidation. Decarboxylation of the Oxalic acid takes place either by conversion of Oxalic acid to Oxalyl CoA, catalyzed by Oxalyl CoA decarboxylase (*OXC*) or directly to CO_2 and formic acid by Oxalate Decarboxylase (*OXDC*). Earlier workers [61] reported purification and partial cDNA cloning of Oxalate Decarboxylase from *Collybia velutipes*. Later, a transgenic tobacco and tomato plants were raised showing expression of *OXDC* gene isolated from fungus *Flammulina velutipes*, especially in fruit [62]. The fruits of transgenic plant showed up to a 90% reduction in Oxalate content, with the concomitant increase in Calcium, Iron and Citrate. A full-length cDNA was isolated using the partial cDNA clone [61] and that cDNA has been successfully expressed in the cytosol and vacuole of transgenic tobacco and tomato plant. The transformation found stable and transferred trait was stably inherited to the next generation. Transgenic tobacco and tomato plants expressing oxalate decarboxylase activity showed remarkable resistance to fungus *Sclerotinia sclerotiorum* that utilizes oxalic acid during infection, which is an additive outcome explaining resistance to fungal infection by the transgenic plants [62-63]. The procedure followed in Tobacco and Tomato is considered as standard protocol to transform vegetable Amaranth. This successful and effective transformation protocol in vegetable Amaranths have consolidated the feasibility of producing transgenic Amaranth with Oxalate decarboxylase gene to lower level of antinutrient Oxalate.

Studies on in vitro response of amaranths

Studies on in vitro response of vegetable Amaranths just have initiated. There are few published reports on tissue cultural practices. An in vitro culture method for both grain and vegetable Amaranths was developed [64] that showed various responses like rapid callus growth, abnormal root formation, development of embryo-like structure from leaf disc. Viable protoplasts isolated from leaf mesophyll of *Amaranthus tricolor* showed expansion and formation of new cell wall. Tissue cultural study in different lines of grain Amaranths highlighted the fact that genotype, growth regulator type and dose, and physiological stage of explants were important parameters for in-vitro regeneration [65]. A protocol for micropropagation of selected genotypes of grain Amaranths and their subsequent exploitation was investigated [66]. These are some of the pioneering works on Amaranth tissue culture forming a foundation for future biotechnological work like in-vitro propagation, germplasm preservation and agronomic improvement through genetic manipulation.

CONCLUSION

In most of the studies, grain Amaranths and conventional vegetable Amaranths were evaluated for nutrient and antinutrient factors, while a large number of crop-wild relatives with almost equal palatability were ignored, which are very essential for sustainable utilization of plant biodiversity. In the present review article, the role of vegetable Amaranths along with their crop-wild relatives, landraces as a source of nutrient and antioxidants have been discussed. There are Few antinutrients lowering the food value which are need to be addressed. Biotechnological approaches have opened up a new avenue for the genetic manipulation to minimize the level of antinutrient elements like Oxalic acid and maximize the level of antioxidants like Squalene. Standardization of a stable and simple *Agrobacterium* – mediated transformation protocol and encouraging achievements in developing transgenic Amaranths with gene of Interest, marker gene or selective gene has further consolidated and brightened the feasibility of that approach.

Acknowledgement

The author expresses his sincere gratitude to Dr. Duilio Iamonico, University of Rome, Sapienza, Italy, for help and cooperation, the Director, Central National Herbarium, Howrah, West Bengal, India, and the Head, Department of Vegetable Crops, Tamil Nadu Agricultural University, Coimbatore for assistance.

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