Review

Vegetable breeding as a strategy of biofortification in carotenoids and prevention of vitamin A deficiency

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Carotenoids are a class of yellow-orange-red pigments distributed in various fruits, spices, herbs and especially in vegetables. These pigments are bioactive components essential to human health. Among the numerous classes of carotenoids, a smaller number is known for having provitamin A activity. This vitamin is essential to the organism in the proper functioning of the vision and the immune system. On the other hand, the deficiency in this vitamin is related to a great number of diseases, which can in severe cases, lead to death. This makes essential the proper supplying of this vitamin to human nutrition. There are two sources from which vitamin A can be derived: (a) vitamin A preformed with retinol esters from animal food sources and (b) from carotenoid found in plant foods. Thus, food fortification is presented as an alternative to reduce vitamin A deficiency. Currently, plant breeding has contributed to the development and introduction of biofortified products. Improved varieties obtained from biofortification have a higher content of nutrients and vitamins, leading to the improvement of human diet. The genetic breeding of vegetables aiming nutritional biofortification is a promising strategy for the increasing of carotenoid concentration in agricultural products and prevention of vitamin A deficiency. Besides the crops that have commonly been used in the biofortification programs, a series of horticulture crops are also promising for insertion in the programs of biofortification in pro vitamin A. Thus, the objective of this review is addressing the problems resulting from vitamin A deficiency and the adoption potential of horticulture species by genetic breeding programs aiming the biofortification in carotenoids.

Key words: Horticulture, hypovitaminosis A, pro vitamin A.

INTRODUCTION

Hunger affects 816 millions of people in the world, resulting in the death of one person at each 3.5 s. Most of the victims of hunger live in developing countries, where 12.9% of the population is undernourished. The
subnutrition and undernourishment result in the deficiency of important micronutrients, mainly vitamin A (Kraemer and Gilbert, 2013). Associated to this, estimates show that nutritional deficiency might be responsible for approximately 45% of the children’s death at the ages under five years (FAO, 2015).

The access to an adequate feeding is a paramount condition in attending the organism needs in components essential to its functioning such as vitamins and minerals. On the other hand, an inadequate feeding can lead to deficiencies in these components. The consumption of foods with pro vitamin A activity at levels lower than that required by the organism constitutes one of the most concerning problems of nutritional deficiency, constituting a worldwide public health problem (WHO, 2009).

The impossibility of synthesis of vitamin A by the organism increases concerning with the deficiency in this vitamin. One of the forms of obtaining this vitamin is through the diet, consuming foods with pro vitamin A activity. The term “pro vitamin A” relates to a series of carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin, which can be biologically transformed into vitamin A (Harrison, 2012).

The clinical levels of vitamin A deficiency (VAD) are related to a great number of abnormalities in the functioning of human organism. The clinical signs of VAD are mainly related to the inadequate functioning of the vision system, as a result of biochemical and functional alterations (xerophthalmia). Such alterations might be progressive, resulting in common visual problems such as nutritional blindness (night blindness), and it might lead to irreversible blindness (Cruz et al., 2005; Mayo-Wilson et al., 2011).

The VAD can cause a series of disorders in the organism, compromising the immunologic system, increasing the vulnerability to diarrheas and infectious respiratory diseases, growth retardation, besides restricting the embryonic development (Chienet et al., 2016; Mamede et al., 2010; Mayo-Wilson et al., 2011; Wiseman et al., 2016).

The strategies of combat to VAD has been based on interventions such as the periodic supplementation of diet with pro vitamin A components, in the fortification, through the addition of massive doses of pro vitamin A components to foods available to population, besides a series of agricultural practices with known effect in the increasing of these components (Graham et al., 2007; Mora et al., 2003).

The biofortification represents one of the main agricultural practice in the combat to VAD. This strategy aims to increase the levels of micronutrients with pro vitamin A activity of basic crops, targeting the improvement of the nutritional status of populations under VAD (Bouis and Welch, 2010).

The programs of biofortification target the assistance of more vulnerable populations through agriculturally sustainable interventions (Pfeiffer and McClafferty, 2007).

Based on traditional methods of crop breeding, the biofortification in a given micronutrient depends on the availability of parental whose edible parts present higher content of micronutrients, on the crossing of these parental and selection of promising progenies for this characteristic (Blair, 2013; de Moura et al., 2014).

The biofortification program of the Consortium of International Agricultural Research Centers (CGIAR), Harvest Plus, represents the main program of biofortification. The multidisciplinary character of Harvest Plus allow it to evaluate numerous aspects inherent to the process of biofortification such as the availability of promising germplasm, the genetic mechanisms determinants of the characteristic of interest, besides the socioeconomic aspects of the populations assisted (Blair, 2013; Bouis and Welch, 2010).

The main crops adopted in the programs of biofortification comprehends rice (Oryza sativa L.), wheat (Triticum aestivum L.), maize (Zea mays L.), cassava (Manihot esculenta C.), bean (Phaseolus vulgaris L.), and sweet potato (Ipomoea batatas L.). These crops have in common essential aspects for the programs of biofortification, such as high yields, high efficiency in reducing nutritional deficiencies in humans, besides a great acceptability by the consumer populations of target regions (Bouis and Welch, 2010).

Besides the crops commonly used in the programs of biofortification, other numerous crops such as winter squash (Cucurbita moschata D.), carrot (Daucus carota L.), and sweet corn (Z. mays L.), are promising for insertion in the programs of biofortification in pro vitamin A (Arscott and Tanumilardjo, 2010; Gallon et al., 2013; O’Hare et al., 2015; Carvalho et al., 2012). Thus, the objective of this review is addressing the problems resulting from vitamin A deficiency and the potential of adoption of horticulture species by genetic breeding programs aiming the biofortification in carotenoids.

**DEFINITION, CLASSIFICATION AND IMPORTANCE OF CAROTENOIDS IN HUMAN HEALTH**

Carotenoids are triterpenes, a class of yellow-orange-red pigments distributed in a series fruits, vegetables, spices and herbs. It is estimated that in developing countries, 70 to 90% of the ingested carotenoids are from fruits and vegetables (Van den Baerg et al., 2000). It is noteworthy that the carotenoids are a family of more than 600 members has been identified in nature, however from these just 60 have provitamin A activity, functioning as precursors of vitamin A in mammals. From the total existing carotenoids, only 20 are present in tissues and in human plasma, of which only six occur in significant quantities: α-Carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and zeaxanthin (Zeraik and Yariwake, 2008).

The human organism does not have the ability to
synthesize vitamin A, thus precursors of this vitamin should be provided to the diet in order to assure adequate levels of this vitamin in the organism. This supply can be achieved by intake of pre formed vitamin A with retinol esters coming from animal foods or by intake of pro vitamin A carotenoids found in vegetable foods (Chagas et al., 2003; Harrison, 2012). Carotenoids provide 25 to 35% of total retinol supply. For developing countries, this contribution can reach up 90% (Van den Baerg et al., 2000).

Regarding the benefits associated with the intake of carotenoids, it effect on health promotion including reducing the risk of developing degenerative diseases such as cancer, cardiovascular diseases cataracts and macular degeneration is highlighted. Studies indicate that they are involved in mechanisms of action related to the carcinogenic metabolism, regulation of cell growth, inhibition of cell proliferation, among others (Rodriguez et al., 2006).

Carotenoid such as α-carotene is described as a suppressor of tumor genesis in the skin, lung, liver and colon, demonstrating activity superior than β-carotene (Gomes, 2007). Lutein is a dihydroxylated carotenoid, which acts as an antioxidant protecting cells from oxidative damage, reducing the risk of development of chronic degenerative diseases (El-Agameyet et al., 2004).

**DISEASES ASSOCIATED TO DEFICIENCIES IN VITAMINS**

**Vitamin A deficiency**

Vitamin A is a fat soluble vitamin, constituent of the organic substances group, soluble in organic solvents and without energetic value (Zancul, 2004). It is essential in the growth, development, maintenance of epithelial tissue, reproduction, adequate functioning of the immune system, and functioning of the visual cycle, in the regeneration of photoreceptors (Ambrósio et al., 2006).

Hypovitaminosis A results from prolonged deficiency of vitamin A. Vitamin A deficiency stands out among the major nutritional problems between most of population in developing countries, having a negative impact on public health. Moreover, it is recognized as a major cause of morbidity and mortality among children in developing countries (Backstrand, 2002).

The World Health Organization (WHO) has included the concept of “vitamin A deficiency” as clinical and subclinical deficiency in this vitamin, when the serum retinol levels are less than 20 μg/dL (0.70 μmol/L). Such condition can leads to adverse health consequences.

Vitamin A deficiency is associated with cases of blindness in approximately 500,000 children every year worldwide, and about half of these children die in this period (Weffort, 2009).

The deficiency in vitamin A reduces resistance to infection, significantly increasing the risk of illness and deaths caused by common childhood infections. In pregnant women, it can increase maternal mortality, the pregnancy risk, besides jeopardizing the lactation. VAD is probably related to the increase of other disorders such as anemia and eye damage. In fact it is estimated that VAD affects approximately 190 million preschool children and 19.1 million pregnant women worldwide (Lira and Dimenstein, 2010).

**Age-related macular degeneration (AMD)**

The age-related macular degeneration is an eye disease that can cause image distortion (microspia and metamorphopsia), spots in the view (relative scotoma) and loss of central vision (absolute scotoma) (Fletcher et al., 2008; Santos et al., 2005). This pathology accounts for approximately 50% of the causes of irreversible blindness (Guimarães and Gerenuuti, 2014).

The macula is the retina pigment responsible for the clear visualization of images. It is identified as a yellow spot observed in the retina center, being a region high visual range. Its yellow color is due to presence of lutein and zeaxanthin, the two carotenoids present in the eyes (Yeum and Russell, 2002).

The density color in the macula has been used as an indicator of a healthy life. The macula selectively accumulates lutein and zeaxanthin in a thin retinal tissue layer that covers this area of the eyes. The protection of the retina by these carotenoids is due to the reduction of oxidative stress and the ability these carotenoids have to filter the light that might be harmful to tissues. These carotenoids have the capacity to absorb 20 to 90% of the blue light that reaches the retina, reducing the extent of photo-oxidative deterioration (Bone et al., 2001; Deli et al., 2004).

The AMD is the main cause of blindness in patients at the ages between 65 and 75 years in developing countries (Anderson et al., 2010). The frequency of AMD in the age group from 55 to 64 years is 13 to 19%, of 31 to 35% in the age group from 65 to 74 years, and 27 to 60% in the group with more than 75 years (Santos et al., 2005). Data from the Brazilian Council of show that approximately 2.9 million Brazilians over 65 years of age have macular degeneration (Stringheta et al., 2009).

There are few options for the treatment of AMD. The measures adopted in the treatment of AMD have low efficiency, especially in stages more advanced. Thus, the intake of foods with vitamins presenting antioxidant activity comprehends a suitable alternative in preventing AMD.

Studies indicate that a diet based on foods rich in carotenoids, especially lutein and zeaxanthin, increase the concentration and density of macular pigment preventing AMD (Serracarbassa, 2006).
STRATEGIES FOR CONTROLLING NUTRICIONAL DEFICIENCIES

Approximately 3 billion of people suffer from micronutrient deficiency worldwide (Rios et al., 2009). Nutritional deficiencies result from high intake of staple foods poor in vitamins and minerals such as rice, corn and wheat and the low consumption of legumes, fruits, vegetables and animal products (Reis et al., 2014). Economically disadvantaged families who do not have resources acquisition of fortified products and populations living in areas with poor soils are more likely to be affected by micronutrients deficiency (Eichholzer, 2003).

Changes such as the industrialization and changes in lifestyle has led to the consumption of foods that provides a lower intake of micronutrients and vitamins (Visioli and Hagem, 2007). The high consumption of processed products, low consume of meat and products nutritionally more rich represent one of the major reasons for the increasing of nutritional deficiencies in developing countries.

In the case of vitamin A deficiency, four strategies has been indicated for its control, namely; (a) balanced diet based on nutritionally rich foods; (b) periodic supplementation with doses of micronutrients; (c) fortification of commonly consumed foods and (d) biofortification (Lee et al., 2000).

Balanced diet

The consumption of nutritionally more rich foods associated food diversification contribute to the proper functioning of the immune system and provides optimal levels of nutrients to organism (Santos et al., 2016). The increase in the consumption of vitamin A rich foods is considered the main long-term strategy of eradication of vitamin A deficiency (Ramalho and Saunders, 2000).

It is important to mention that achieving a balanced diet requires the establishment of marketing plans, aiming to provide information to the target public. It is important to consider that an inadequate supply of vitamin A is directly related to eating habits (Ramalho and Saunders, 2000).

Supplementation

It consists in the supply of nutrients, generally encapsulated or in the form of powder, which is added to the foods. The supplement dosage should consider the profile of the population, the nutritional status and frequency in which the foods to be supplemented are consumed.

The massive supplementation with vitamin A for children at the ages of 6 to 59 months and women after childbirth is considered an emergency strategy of intervention in combating vitamin A deficiency (Queiroz et al., 2013). In fact, in regions where this disease is endemic, it significantly contributes to the reduction of mortality of children under five years of age (Martins et al., 2007).

The distribution of high doses of vitamin A, in conjunction with the National Immunization Program, made Brazil the pioneer in this initiative, which is now recommended by WHO in several countries as an efficient procedure for combating vitamin A deficiency (Rodrigues and Roncada, 2010).

Fortification

Food fortification, which comprehends the addition of vitamins and minerals to foods, is the most economical, flexible and socially acceptable way to improve the nutritional status of people in developing countries (Backstrand, 2002). This strategy offers several advantages, especially the high coverage of population, the fact it does not change the eating habits besides presenting low risk of toxicities (Zancul, 2004).

The WHO recognizes a series of forms of food fortification. One of them is the “mass fortification or universal”, which consists of adding micronutrients to foods consumed by the vast majority of the population, being regulated by governments; the "fortification in open market", which comprehends the fortification of food by industry on its own initiative and “Directed fortification”, which is the fortification of foods eaten by groups at high risk of nutritional deficiency (Ferreira et al., 2015).

Biofortification

Biofortification involves plant breeding techniques aiming the development of improved varieties and the providing of agricultural products with higher concentrations of bioavailable minerals and vitamins. This strategy helps to correct, eliminate or prevent diseases caused by nutritional deficiency efficiently (de Oliveira et al., 2009). It is considered as a new concept for agriculture and a tool to improve human health sustainably (Bouis, 1999).

The process of bio-fortification presents a series of advantages, as the ongoing benefits at costs lower than those related to the supplementation and fortification; it does not cause changes in the eating habits of consumers neither changes the agricultural practices; It does not drastically affect the taste, texture, appearance or the preparation of foods; It provides a high coverage of populations, with low risk of toxicity, besides replacing the use of dietary supplements (Bouis 1999; Lima Neto, 2013).

The Consultative Group for International Agricultural Research (CGIAR) began to prioritize its crop breeding project for nutritional improvement of foods from 2002. In 2004, the group launched the Challenge Program in
Biofortification Harvest Plus. This program aims to improve the nutritional quality of the main crops adapted to marginal areas of the world in order to ensure the diet enrichment of poorest people. In 2005, the Bill Gates and Melinda Gates Foundation began supporting part of this project (CGIAR, 2012; Hirschi, 2009; Pfeiffer and McLafferty, 2007; Sayre et al., 2011).

The breeding of plants aiming nutritional biofortification should consider important aspects such as the program capacity of manipulating characteristics associated with nutritional quality. The process of biofortification should prioritize the combining of characteristics as high nutritional content with agronomic aspects of interest, assure the ready availability of nutrients after traditional preparation of foods, besides assuring that the biofortified foods have a good acceptability by consumers (Mezette et al., 2009; Pfeiffer and McLafferty, 2007; Welch and Graham, 2004; Zancul, 2004).

The biofortification with pro-vitamin A carotenoids has been considered the main strategy to reduce vitamin A deficiency and AMD in developing countries and its implementation is closely linked to the genetic breeding programs of crops.

**VEGETABLES FOUNT OF PRO VITAMIN A CAROTENOIDS**

Researches indicates the existence of a wide genetic variability for the concentration of pro vitamin A carotenoids present in edible parts of crops such as pumpkin, watercress, lettuce, sweet potatoes, broccoli, cashews, carrots, chicory, kale, spinach, guava, papaya, cassava, mango, corn, pumpkin and mustard (Table 1). This variation is an important resource for breeding programs, particularly in conventional breeding (Rios et al., 2009; Souza and Boas, 2002).

**Winter squash (C. moschata Duch.)**

Winter squash (C. moschata Duch.) is one of the major sources of pro-vitamin A carotenoids, especially β-carotene, α-carotene and lutein. Besides its socioeconomic importance, varieties of this crop have a wide environmental adaptability and show high variability in relation to commercial characteristics. Although this crop presents a good acceptability for consumers and potential for expansion of its cropping area, the works aiming the breeding of this crop are still scarce (Gonçalves et al., 2015).

The germplasm belonging to the Brazilian diversity center and other countries have enormous variability as to total content of carotenoids and agronomic characteristics, allowing the selection of promising genotypes for breeding programs aiming the biofortification of this crop (Lima Neto, 2013).

Researches show that β-carotene is the predominant carotenoid in C. moschata, followed by α-carotene and lutein. Evaluating 55 accessions of C. moschata belonging to the Vegetable Gene Bank of the Federal University of Viçosa, Lima Neto (2013), identified in this germplasm variations of 12.8 to 286.7 μg g⁻¹ for β-carotene, of 8.1 to 153.8 μg g⁻¹ for α-carotene and of 0.004 to 1.32 μg g⁻¹ for lutein. Similar results were found by González et al. (2001) with the cultivar Butternut, in which the predominance of β-carotene was also observed.

**Sweet potato (I. batatas L.)**

Sweet potato has high rusticity and a broad spectrum of use. This crop has a great economic importance, especially in developing countries (Cardoso et al., 2005). This crop presents great genetic variability allowing the selection of genotypes for many purposes such as pest resistance, disease and stress resistance and better nutritional quality (Neto et al., 2011).

Sweet potato is considered a source of calories, minerals and vitamins such as A, C and of complex B, besides presenting roots rich in calcium, potassium and carbohydrate. According to Alves et al. (2012) the genotypes of sweet potato show high variability in β-carotene content (<1 to 130 μg g⁻¹), indicating potential gains for biofortification in this component through genetic breeding.

White or cream pulp varieties have low levels of

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**Table 1. Variation in carotenoids in different vegetables crops.**

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Components (μg g⁻¹)</th>
<th>References</th>
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<tbody>
<tr>
<td></td>
<td>α</td>
<td>β</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>153.8</td>
<td>286.7</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>291.1</td>
<td>286.7</td>
</tr>
<tr>
<td>Carrot</td>
<td>62.2</td>
<td>70.3</td>
</tr>
<tr>
<td>Cassava</td>
<td>33.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Corn</td>
<td>4.9</td>
<td>5.5</td>
</tr>
</tbody>
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α, alfa carotene; β, beta carotene; L, lutein.
vitamins, since they show starch as their main content. On the other hand orange pulp cultivars are rich in provitamin A. The pulp of sweet potato processed into flour can partially replace wheat flour in the preparing of cakes, cookies and other products used in school feeding (Alves et al., 2012).

**Carrot (D. carota L.)**

Carrot (D. carota L.) is one of the most important vegetable crops, due to its large consumption worldwide and the acreage extension (Lima et al., 2001).

This crop is considered one of the major vegetable sources of pro vitamin A carotenoids, especially α and β-carotene. According to Pigoli et al. (2014) varieties of this crop such as Nantes shows on average 50% of β-carotene, 30% of α-carotene and 5% of γ-carotene of total carotenoids.

The carrot dark orange cultivars present higher accumulation of pro vitamin A carotenoids, presenting on average 138.0 μg g⁻¹ of total carotenoids and prevalence of β-carotene (70.3 μg g⁻¹) in relation to α-carotene (62.3 μg g⁻¹) (Vieira et al., 2012).

**Cassava (M. esculenta C.)**

Cassava (M. esculenta C.) is cultivated in tropical and subtropical regions of Asia, Africa and Latin America and is considered the third largest source of calories in the tropics, surpassed only by rice and corn. Its consumption has doubled from 24 million tons to 58 million tons over the past 30 years (Konjalves et al., 2015).

Currently, the breeding programs of table cassava have focused on developing varieties that have agronomic characteristics of interest associated with its biofortification. The biofortification of cassava prioritizes the increasing of carotenoids such as β-carotene in yellow staining roots and lycopene in pink color roots (Carvalho et al., 2012; Carvalho et al., 2011).

Cassava presents a wide genetic diversity (Gomes et al., 2016), including the carotenoid content. Da Silva et al. (2014) identified genotypes with a carotenoid content varying from 1.47 to 19.18 μg g⁻¹ of fresh weight, where the pink colored roots presented highest carotenoid levels in relation to yellow and cream pulp varieties. This characteristic allows the screening and selection of promising and contrasting genotypes of cassava as to the content of carotenoids.

**Sweet corn (Z. mays L.)**

Maize (Z. mays L.) is a versatile food and can be consumed as grain or vegetable, being consumed in the form of cooked cob, canned and also in the preparation of traditional dishes. In the United States and Canada sweet corn is one of the vegetables highly appreciated by the population (Bordalvo et al., 2005).

Sweet corn is an excellent food because of its nutritional composition. It has about 1,290 kcal kg⁻¹, 3.3% of protein, 27.8% of carbohydrates and only 0.8% of fat (Barbosa, 2013).

The breeding programs of sweet corn in Brazil for example, aim the development of varieties biofortified in Fe, Zn and provitamin A. These varieties are intended to populations with nutritional deficiencies such as iron and vitamin A deficiencies (Kurilich and Juvik, 1999).

Traditional varieties of sweet corn have low levels of provitamin A, with concentrations between 0.13 and 2.7 μg g⁻¹ for β-carotene, of 0 and 1.3 μg g⁻¹ for α-carotene and 0.13 and 1.9 mmol g⁻¹ for β-cryptoxanthin (Kurilich and Juvik, 1999). Genetic breeding of sweet corn has enabled the obtaining of varieties with concentration of pro vitamin A higher than 13 μg g⁻¹ (Cardoso et al., 2009).

When assessing the genetic diversity among corn genotypes as to carotenoid content, Rios et al. (2011), obtained averages of 15.38 to 26.90 μg g⁻¹ for total carotenoids and of 1.50 to 2.32 μg g⁻¹ for pro vitamin A carotenoids. This indicates the possibility of increasing the content of these components through genetic breeding.

**Conclusion**

The prevalence of vitamin A deficiency implies in negative consequences in human health. The genetic breeding of vegetables aiming nutritional biofortification is a promising strategy for the increasing of carotenoid concentration in agricultural products, improving the diet of human health. The programs of biofortification should be based in the achievement of quantitative and qualitative gains, aiming a nutritionally adequate feeding of populations.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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