Breeding Vegetables with Improved Bioactive Properties

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Abstract

Vegetable crops contain significant amounts of many bioactive compounds which prevent and/or protect against chronic diseases. Consumers increasingly demand vegetables with improved bioactive properties and this is stimulating the development of new cultivars with enhanced content in bioactive compounds. Generally, breeding programmes of specific crops are aimed at increasing the most relevant bioactive compounds of each crop. The success of these breeding programmes depends on the availability of sources of variation for bioactive compounds. Traditional varieties and wild relatives collections are generally very variable for these compounds and in many cases it is possible to identify sources of variation of great interest among these materials. There are several breeding strategies for improving the content in bioactive compounds, including conventional strategies based on phenotyping, as well as modern strategies that rely on marker assisted selection or genetic transformation. Breeding for the enhancement of bioactive compounds may affect vegetables in a positive (e.g., extended shelf-life) or negative (e.g., browning, bitterness) way other relevant traits for the success of a cultivar. The negative side effects may be circumvented by using complementary breeding strategies aimed at reducing or removing the negative impact on the characteristics and performance of a new cultivar. In summary, breeding can contribute to the development of a new generation of vegetable crops with enhanced bioactive properties and therefore to the development of the horticultural sector.

Keywords: *antioxidants, breeding strategies, diversity, genetic improvement, marker assisted selection, new cultivars, wild relatives*

INTRODUCTION

Many epidemiological studies reveal that people having a high level of consumption of vegetables presents a better health and lower risk of chronic diseases, including cardiovascular diseases and different types of cancer (Hung *et al.*, 2004; Boeing *et al.*, 2012). Vegetables contain many bioactive compounds and represent a major source of antioxidants and other compounds that are beneficial to human health (Terry, 2011; Rajarathnam *et al.*, 2014). Consumers are increasingly demanding vegetables with bioactive properties that contribute to maintaining a good health and preventing diseases (Weatherspoon *et al.*, 2014). In consequence, breeding programmes in vegetables are increasingly considering the content in bioactive compounds as a major breeding objective (Diamanti *et al.*, 2011).

In many vegetable crops, breeding programmes have been devoted to improving yield, resistance to diseases, produce uniformity or apparent quality (Prohens and Nuez, 2008a, 2008b). Other important traits, like those related to organoleptic quality have generally been considered of second rank compared to breeding for yield, although in some crops breeding for organoleptic quality has also been considered an important trait in breeding programmes (Casañas and Costell, 2006). The content in bioactive compounds has been usually considered of low priority in breeding programmes, and few cultivars have been developed having dramatically improved contents in bioactive compounds. Among them, some new varieties have been released that are characterized (and are advertised as such) with a higher conent in bioactive compounds. Among them there are some prominent examples, like the 'Fashion' watermelon, which has a high content in lycopene and citrulline, the 'Lycomate' and 'Doublerich' tomatoes, which have, respectively, a high content in lycopene and vitamin C, the Almagro eggplant, with high contents in chlorogenic acid (Watada *et al.*, 1986; Tarazona-Díaz *et al.*, 2011; Hurtado *et al.*, 2014). As a consequence of this increased content in bioactive constituents these vegetable varieties have a high added value and reach a higher price in the market.

Given the increased demandand by consumers for vegetables with increased content in bioactive compounds, researchers and breeders are developing new knowledge and tools for an efficient breeding of the content in bioactive compounds in vegetables (Cámara, 2006; Diamanti *et al.*, 2011). In this way, there is an increasing number of breeding programmes and scientific studied aimed at improving the content in bioactive compounds of vegetables, and the trend seems that will continuing in the coming years. In this respect, the development of genomics is greatly contributing to improve marker assisted selection as well as to develop tools for an efficient breeding (Pérez-de-Castro *et al.*, 2012).

In this paper we deal with some relevant issues related to breeding for the content in bioactive compounds in vegetables, including breeding objectives, diversity and sources of variation, breeding strategies, and collateral effects on other traits of interest for the success of a cultivar. The objective is provide general and comprehensive information for the development of vegetables with improved bioactive properties.

BREEDING OBJECTIVES FOR IMPROVING BIOACTIVE PROPERTIES

Plant breeding is aimed at exploiting the genetic potential of plants for benefit of humans (Rodríguez-Burruezo *et al.*, 2009; Acquaah, 2012). Therefore, breeding programmes aimed at improving the bioactive properties of vegetables will be devoted to developing new varieties with contents of bioactive compounds higher than those of the predominant varieties (Cámara, 2006; Diamanti *et al.*, 2011). In this respect, breeding efforts can be devoted to improving a specific compound (e.g., chlorogenic acid, β-carotene, glucoraphanin, etc.), a group of compounds (e.g., total phenolics, total carotenoids, total glucosinolates, etc.), or an aggregate property (e.g., antixodant activity, anticarcinogenic activity, etc.). Each of these levels has different levels of complexity from the point of view of breeding. Breeding for specific compounds generally will be less complex from the genetic point of view than breeding for groups of compounds or aggregate properties, in which the genetic control is usually more complex (Rodríguez-Burruezo *et al.*, 2009; Acquaah, 2012).

Within vegetable crops there are many compounds with bioactive properties, like phenolics, carotenoids, glucosinolates, vitamins, folates, phytosterols, etc. (Cámara, 2006; Rajaranthnam *et al.*, 2014). However, each of these groups contain many compounds, and there are important differences in the activity of individual compounds within each group (Ignat *et al.*, 2011; Fernández-García *et al.*, 2012). Also, given that there are important differences among vegetables in the compounds responsible for the bioactive properties (Cámara, 2006; Tsao *et al.*, 2006; Prohens and Nuez 2008a, 2008b), breeding programmes are usually directed to increasing the levels of those compounds or groups of compounds that are responsible of the most relevant properties for each vegetable crop (Table 1).

DIVERSITY AND SOURCES OF VARIATION

As occurs with any breeding programme, the success of a breeding programme for improving the bioactive properties of a vegetable crop requires having genetic diversity available for the target trait/s (Rodríguez-Burruezo *et al.*, 2009; Diamanti *et al.*, 2011; Acquaah, 2012). Identification of genetic diversity in collections of germplasm or populations for bioactive compounds can be done using conventional methods based on classical genetics and quantitative genetics methods or with modern biotechnologies (Rodríguez-Burruezo *et* *al.*, 2012; Acquaah, 2012; Pérez-de-Castro *et al.*, 2012).

As occurs with nutrients (e.g., carbohydrates, proteins, minerals, etc.) in which modern breeding has led to the undesirable effect of "dilution of nutrients" (Davis, 2009), for bioactive compounds there has also been a reduction in the levels in modern varieties when compared with traditional varieties. In this way, increases in yield have frequently been associated to a reduction in the content of compounds with bioactive properties. Similarly, the introduction of long shelf-life genes, which alter ripening, may produce a reduction in the content of bioactive compounds. In this respect, in the case of tomato, gene *rin*, which is present in many long shelf-life varieties of

Tab. 1. Some important vegetable crops and major bioactive groups of compounds and specific compounds for which breeding programmes are being performed.

Vegetable crop	Compounds with bioactive properties	References
Artichoke (*Cynara cardunculus* var. *scolymus* L.)	Phenolics, in particular chlorogenic acid	Pandino *et al.* (2012)
Asparagus (*Asparagus officinalis* L.)	Phenolics, in particular phenolic acids, flavonoids, flavanols and ascorbic acid	Lee *et al.* (2014)
Cabbage and cauliflower (*Brassica oleracea* L.)	Glucosinolates, cartoenoids and anthocyanins	Padilla *et al.* (2007)
Carrot (*Daucus carota* L.)	Carotenoids and phenolics, in particular cholorogenic acid and anthocyanins	Baranski *et al.* (2012)
Celery (*Apium graveolens* L.)	Phenolics	Yao *et al.* (2010)
Cucumber (*Cucumis sativus* L.)	Carotenoids, in particular β-carotene	Navazio and Simon (2001)
Eggplant (*Solanum melongena* L.)	Phenolics, in particular chlorogenic acid and antocyanins	Prohens *et al.* (2007)
Leek (*Allium porrum* L.)	Phenolics, lutein, β-carotene, ascorbic acid and vitamin E	Bernaert *et al.* (2012)
Lettuce (*Lactuca sativa* L.)	Carotenoids, in particular β-carotene and lutein, and anthocyanins	Mou (2005)
Melon (*Cucumis melo* L.)	Carotenoids	Harel-Beja *et al.* (2010)
Onion (*Allium cepa* L.)	Phenolics, in particular flavonoids, flavonols and anthocyanins, and ascorbic acid	Yang *et al.* (2004)
Pepper (*Capsicum annuum* L.)	Carotenoids, phenolics, and ascorbic acid	Rodríguez-Burruezo *et al.* (2011)
Pumpkin, squash and zucchini (*Cucurbita* spp.)	Carotenoids, tocopherol, ascorbic acid	de Carvalho *et al.* (2012)
Spinach (*Spinacia oleracea* L.)	Lutein and phenolics	Pandjaitan *et al.* (2005)
Table beet (*Beta vulgaris* subsp. *vulgaris* L.)	Betalains	Gaertner *et al.* (2005)
Tomato (*Solanum lycopersicum* L.)	Carotenoids, in particular lycopene, phenolics, and ascorbic acid	Adalid *et al.* (2010)
Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)	Carotenoids, in particular lycopene, and ascorbic acid	Yoo *et al.* (2012)

tomato (Marín, 2013), causes a reduction in the content in lycopene in the fruit (Vrebalov *et al.*, 2002). This indicates that very often breeding programmes aimed at improving the bioactive properties of vegetables will need to identify sources of variation in materials other than élite modern varieties. Furthermore, modern varieties usually have a narrow genetic base (Simmonds, 1997; Rodríguez-Burruezo *et al.*, 2009; Acquaah, 2012) and in order to improve the bioactive properties breeders very frequently will have to turn to materials like traditional varieties and wild relatives.

Traditional varieties usually present a high variation for the content in bioactive compounds, which values much higher than those of modern commercial varieties (Koch and Goldman, 2005; Mou, 2005; Rodríguez-Burruezo *et al.*, 2005; Burger *et al.*, 2006; Prohens *et al.*, 2007; Perkins-Veazie *et al.*, 2010). Traditional varieties have the advantage that hybridizations with modern élite materials are fertile and hybrids and subsequent generations present the typical characteristics of the domesticated species (Rodríguez-Burruezo *et al.*, 2009). On occasion, related wild species represent an additional source of variation of great interest as they present values much higher (frequently several times higher) than those present in the cultivated species (Willits *et al.*, 2005; Prohens *et al.*, 2013). However, in these cases, breeding programmes can encounter some difficulties in hybridization, hybrid sterily or reduced fertility, and the need of high number of backcross generations to remove the undesirable part of the genetic background of the donor wild relative (Kalloo and Chowdhury, 1992; Rodríguez-Burruezo *et al.*, 2009). In any case, the availability of adequate sources of variation usually requires the screening of large germplasm collections in order to identify materials of interest (Rodríguez-Burruezo *et al.*, 2005; Prohens *et al.*, 2007). Once these sources of variation have been identified, an adequate and efficient breeding strategy has to be applied in order to introgress it into an appropriate genetic background in order to obtain a commercially valuable cultivar (Simmonds, 1997; Rodríguez-Burruezo *et al.*, 2009; Acquaah, 2009).

BREEDING STRATEGIES

Although some bioactive properties of specific vegetable crops may be qualitative (i.e., presence/absence), in most cases the traits responsable of the bioactive properties are quantitative. Also, apart from genetic differences, the high environmental influence in the expression of this type of traits favours the existence of continuous variation, even when the trait has an oligogenic control (Tsao *et al.*, 2005). This implies that usually strategies for breeding for bioactive properties are those used for quantitative traits. Depending on the type of strategy to be used we can distinguish between conventional strategies based on phenotyping, marker assisted selection, and strategies derived from genetic transformation (Gepts, 2002; Collard and Mackill, 2008).

Conventional strategies are based on selection in genetically variable populations for the trait of interest and on hybridization and selection in segregating generations (Rodríguez-Burruezo *et al.*, 2009; Acquaah, 2012). The success of plant breeding in the XXth centtury have mostly relied on these conventional strategies, which have proved highly successful and efficient for yield traits (Pérez-de-Castro *et al.*, 2012). Application of these breeding methods to traits related to bioactive properties shows that for these traits it is posible to achieve important genetic advances. For example, we have found that in eggplant the narrow-sense heritability for total phenolics was of 0.5 (Prohens *et al.*, 2007), which together with the wide diversity for this trait in the germplasm collections indicates that it is possible to achieve considerable genetic advances for this trait (Plazas *et al.*, 2013).

The increasing availability of molecular and genomic tools is fostering, as occurrs with other traits, a revolution in breeding for bioactive properties (Pérez-de-Castro *et al.*, 2012). In this way, thanks to the new developments it has been posible to identify quantitative trait loci (QTL) as well as genes and allelic variants of these genes involved in the synthesis of compounds responsible for bioactive properties as well as molecular markers linked to them (Just *et al.*, 2009; Kinkade and Foolad, 2013a; Sotelo *et al.*, 2014). This makes feasible in vegetable crops the marker assisted selection for traits related to bioactive properties (Kinkade and Foolad, 2013b; Plazas *et al.*, 2013). Therefore, once the genes or QTL involved in the target bioactive compound/s are identified selection can be done of the individuals of interest without the need of phenotyping (Collard and Mackill, 2008). This strategy can also be very useful for gene pyramiding for different favourable alleles involved in the biosynthetic pathways of the target compounds (Ishii and Yonezawa 2007a, 2007 b; Plazas *et al.*, 2013).

The improvement in the content of bioactive compounds can also be achieved by means of genetic transformation, which allows important increases in a short period of time (Díaz de la Garza *et al.*, 2007; Guo *et al.*, 2012). Genetic transformation requires the introduction using different transformation techniques of one or several genes from different organisms in the genome of the target species in order to achieve transgenesis (Kole *et al.*, 2010). However, transgenic varieties are suffering from an important rejection at the social level and it seems difficult that they represent at a short-medium term a realistic alternative for the development of commercially accepted variaties, at least in Europe (nicolia *et al.*, 2014). Cisgenesis, which consists in the genetic transformation resulting only in the introduction of genes obtained from materials sexually compatible with the donor variety (Jacobsen and Schouten, 2007), is an alternative that is free from most of the critics of transgenesis. However, given that cisgenesis uses genetic transformation techniques its utilization is not free of criticism and it is unlikely that it becomes approved soon in Europe.

EFFECTS OF BREEDING FOR BIOACTIVE PROPERTIES ON OTHER TRAITS

The success of a new cultivar requires that all the actors involved in the chain that goes from the production to the consumer become satisfied with the performance of the new variety (Rodríguez-Burruezo *et al.*, 2009; Acquaah, 2012). In this respect, the improvement in the content of bioactive compounds, apart from an increase in the compound/s of interest may have other colateral effects, which can be positive or negative, on other agronomic or quality traits that may affect the success of the new cultivar.

An example of a positive effect is the increase in the shelf-life of tomato fruits with high levels of anthocyanins in the fruit (Zhang *et al.*, 2013). In this respect, the antioxidant properties of many bioactive compounds may have a role in extending shelf-life, as they are able to neutralize the free radicals that are generated during the periods of senescence or as a consequence of infection (Davey and Keulemans, 2004; Singh *et al.*, 2010; Zhang *et al.*, 2013). Regarding negative effects, the increase in phenolics content can result in an increase of browning in vegetables like artichoke or eggplant (Prohens *et al.*, 2007; Cefola *et al.*, 2012). However, selection of allelic variants of polyphenol oxidases (necessary for the development of enzimatic browning) with reduced activity makes possible the selection of varieties with high content in phenolics and low browning (Plazas *et al.*, 2013; Chi *et al.*, 2014). Another example of a negative effect associated to the increase in bioactive compounds corresponds to glucosinolates, which have a bitter flavour, in brassicas (Drewnowski and Gomez-Carneros, 2000). In this case, the perception of the bitter flavour for different glucosinolates is different (Williams and Pun, 2010), and with positive selection for glucosinolates with low bitterness and negative selection for glucosinolates with high bitterness it might be possibile to improve the content in glucosinolates without increasing bitterness (Wricke and Weber, 1986). These two examples show that there are strategies that allow combining an increase in the content in compounds with bioactive properties and reduce the undesirable effects on other traits important for the success of a cultivar.

CONCLUSIONS

Breeding for bioactive properties in vegetables is increasingly becoming important in breeding programmes in vegetable crops. There are many bioactive compounds in vegetables and, therefore, there are many possibilities for the development of new cultivars with improved bioactive properties. The utilization of a wide diversity in breeding programmes, in particular from traditional varieties and wild relatives, on which applying adequate strategies for increasing the content of bioactive compounds will lead to the development of new vegetable crops cultivars with improved bioactive properties compared to present cultivars. At the same time, these strategies will strengthen the positive effects of the increase in these bioactive compounds on other traits of agronomic or comercial interest and to reduce the negative effects that may have on other characteristics. In summary, breeding for bioactive properties will allow the development of a new generation of cultivars with improved bioactive properties.

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