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Breeding for Chlorogenic Acid Content in Eggplant: Interest and Prospects

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Abstract

Chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA) is an ester of caffeic acid and (-)-quinic acid with many beneficial properties for human health, such as anti-oxidant, anti-inflammatory, cardioprotective, anti-carcinogenic, anti-obesity, and anti-diabetic properties. This has raised an interest for the development of new crop cultivars with increased CGA content. One of the crops with higher CGA content is eggplant (*Solanum melongena*). There is a wide diversity for CGA content in cultivated eggplant germplasm, which is influenced by the fruit developmental stage, storage conditions, and environmental factors. Therefore, appropriate experimental designs are required for an efficient breeding. Several strategies are proposed for breeding for high CGA content such as intraspecific variation, selection among accessions, development of hybrids and lines with good agronomic and commercial characteristics, or introgression of the high CGA trait in élite lines. Some wild relatives, like *S. incanum*, present higher CGA contents than those of eggplant. Interspecific hybridization can be used to introgress favorable alleles from the wild species into the genetic background of cultivated eggplant. Fruit flesh browning, as a result of CGA oxidation by polyphenol oxidases, could be a side effect of increasing the CGA content in eggplant. However, experimental results indicate that the relationship between CGA content and fruit flesh browning is low or moderate. Furthermore, selection for low polyphenol oxidase activity might result in reduced fruit flesh browning. Overall, the available data suggest that the development of eggplant cultivars with improved functional quality resulting from a higher CGA content is feasible.

Keywords: functional properties, germplasm, hybridization, polyphenol oxidase, *Solanum incanum*, *Solanum melongena*

1. What is chlorogenic acid?

Chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA) is a phenolic compound resulting from the esterification of caffeic acid and the aliphatic alcohol (–) quinic acid (1L-1(OH)-3,4/5-tetrahydroxycyclo-hexane carboxylic acid) (Fig. 1). CGA is present in many plants where it plays a role in plant defense, as well as an antioxidant (Korkina, 2007; Leiss *et al.*, 2009; Ngadze *et al.*, 2012). The broader term "chlorogenic acids" has also been used to refer to a family of esters formed between certain *trans*-cinnamic acids (caffeic, ferulic and *p*-coumaric acids) and quinic acid (Clifford, 2000). The main subgroups of chlorogenic acids include: mono-esters of caffeic acid (caffeoylquinic acids, *p*-coumaroylquinic acids and feruloylquinic acids), di-esters, tri-esters, a single tetra-ester of caffeic acid, and mixed di-esters of caffeic and ferulic acid (caffeoylferuloylquinic acids) or caffeic and sinapic acid (caffeoylsinapoylquinic acids). Mixed esters involving various permutations of between one and three residues of caffeic acid with one or two residues of a dibasic aliphatic acid (such as glutaric, oxalic, succinic) have also been denominated chlorogenic acids (Clifford, 2000). However, for the purposes of the present paper we use the term chlorogenic acid (CGA) to refer specifically to 5-*O*-caffeoyl-quinic acid.

CGA is included in the broad category of polyphenols, which are typically classified into one of either two categories: flavonoids and phenolic acids (Macheix, 1990). Among the latter, hydroxycinnamic acids, of which CGA is a major representative, is considered the main class.

2. The interest for breeding for CGA content: bioactive properties

Dietary polyphenols from numerous plant species have shown to be beneficial for human health due to its known biological activities, which include free-radical scavenging, regulation of enzymatic activity, and modulation of several cell signaling pathways (Sato *et al.*, 2011). In fact, many of them are being actively studied as potential treatments for various metabolic and cardiovascular diseases. For example, resveratrol from red wine, epigallocathechin-3-gallate from green tea, curcumin from turmeric, and quercetin from different sources have all been studied as potential therapeutic agents to induce weight loss, lower blood pressure, attenuate glucose levels and insulin resistance, and improve hemoglobin A1c and lipid profile in humans (Andújar *et al.*, 2012).

CGA is found in many edible and medicinal plants, and is well known for having various biological properties

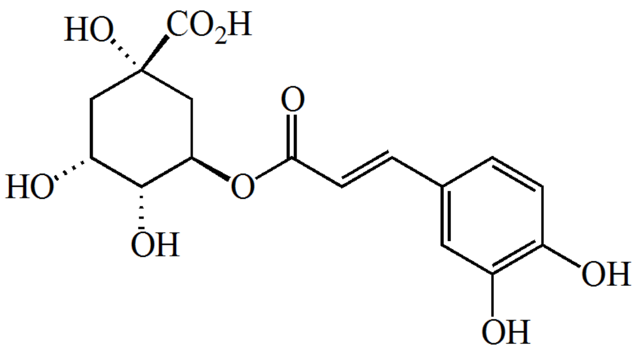

Fig. 1. Chemical structure of chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA)

of interest for human health. These include anti-oxidant, anti-inflammatory and analgesic properties demonstrated both *in vitro* and *in vivo* (dos Santos *et al.*, 2006; Jin *et al.*, 2006; Morishita and Ohnishi, 2001; Sato *et al.*, 2011; Sheu *et al.*, 2009), as well as strong anti-microbial activity (Almeida *et al.*, 2006). In relation to this anti-oxidant/anti-inflammatory activity, several studies also highlight CGA neuroprotective (Ahn *et al.*, 2011) and cardioprotective (Chen *et al.*, 2009; Zhao *et al.*, 2012) effects.

A number of animal studies have indicated that CGA is hypotensive (Suzuki *et al.*, 2002, 2006). This blood pressure-lowering activity also occurs in humans, as confirmed by clinical trials: the administration of 140 mg/day of CGA to mildly hypertensive subjects decreased both systolic and diastolic blood pressure significantly (Watanabe *et al.*, 2006).

CGA is also known to exert selective anti-carcinogenic effects via induction of apoptosis in many human cancer cells, such as leukemia cells (Yang *et al.*, 2012) and lung cancer cells (Burgos-Morón *et al.*, 2012). Other biological activities of CGA include its anti-obesity effect with improvement of lipid metabolism (Cho *et al.*, 2010), and a delay in intestinal glucose absorption and inhibition of gluconeogenesis (Ong *et al.*, 2012), which contributes to an anti-diabetic effect (Coman *et al.*, 2012).

CGA is one of the most abundant polyphenols in the human diet and is highly bioavailable in nature (dos Santos *et al.*, 2006). This fact, together with its numerous bioactive properties potentially beneficial for human health, encourages the use of breeding approaches in order to increase its level in food crops (Niggeweg *et al.*, 2004).

3. Eggplant as a source of CGA in the diet

The major dietary sources of CGA are vegetables, fruits, and beverages like coffee (Azuma *et al.*, 2000). It is estimated that humans consume up to 1 g of CGA per day (Chen *et al.*, 2009). Although coffee is considered a major source of CGA in the human diet, as regular coffee drinkers may consume up to 0.5 - 1 g of CGA per day (Olthof *et al.*, 2001), fruits and vegetables also make a substantial contribution to CGA intake (Olthof *et al.*, 2001). In this respect, eggplant is one of the vegetables with a higher content in CGA (Tab. 1).

CGA is, by far, the major phenolic compound of the eggplant fruit, and typically makes between 80% and 95% of the total hydroxycinnamic acids present in the fruit flesh (Prohens *et al.*, 2013; Stommel and Whitaker, 2003; Whitaker and Stommel, 2003). Also, it has been found that concentrations of CGA in the eggplant fruit skin are similar to those present in the fruit flesh (Gajewski *et al.*, 2009). When compared with the estimation of the total phenolics content by means of the spectrophotometric method of Folin-Ciocalteu, CGA typically represents between 30% and 75% of the total phenolics of the fruit when harvested at the commercially mature stage (Luthria, 2012; Mennella *et al.*, 2012). CGA content in eggplant flesh is highly correlated with total phenolics and antioxidant activity, with r^2 values of 0.87 and >0.95, respectively (Luthria *et al.*, 2010, 2012). These results confirm that CGA is the most relevant phenolic compound in the eggplant fruit, and the major contributor to the high antioxidant capacity of eggplant. In fact, eggplant ranks among the vegetables with highest oxygen radical absor-

Tab. 1. Comparison of contents in chlorogenic acid (5-*O*-caffeoyl-quinic acid; CGA) in eggplant with other major vegetables, fruits, and plant products providing significant amounts of CGA to the diet

Plant source	CGA ($g \cdot kg^{-1}$ dw)	References
Eggplant	4.9-21.6	Stommel and Whitaker (2003)
	4.2-9.5	Whitaker and Stommel (2003)
	1.5-2.2	Gajewski *et al.* (2009)
	5.0-8.1	Singh *et al.* (2009)
	2.6-6.7	Luthria *et al.* (2010)
	11.2-24.0	Mennella *et al.* (2010)
	1.4-8.4	Luthria (2012)
	14.1-28.0	Mennella *et al.* (2012)
Other vegetables		
Artichoke	1.1-1.8	Lutz *et al.* (2008)
Carrot	0.3-18.8	Sun *et al.* (2009)
Pepper	0.7-0.9	Hallmann and Rembialkowska (2013)
Tomato	0.2-0.4	Hallmann (2012)
Fruits		
Apple	0.4-1.2	van der Sluis *et al.* (2001)
Apricot	0.02-0.51	Madrau *et al.* (2009)
Cherry	0.02-0.09	Serra *et al.* (2011)
Peach	0.1-1.6	Andreotti *et al.* (2008)
Plum	0.4	Khallouki *et al.* (2012)
Other plant products		
Coffee	27.9-52.0	Monteiro and Farah (2012)
Mate tea	4.8-24.9	Heck *et al.* (2008)
Potato	0.01-4.60	Deußer *et al.* (2012)
Sunflower seeds	29.9-45.5	Singh *et al.* (1999)

bance capacity due to its high content in phenolics (Cao *et al.*, 1996).

The multiple health benefits of eggplant, which include anti-oxidant, anti-diabetic, hypotensive, cardioprotective, and hepatoprotective effects (Akanitapichat *et al.*, 2010; Das *et al.*, 2011; Kwon *et al.*, 2008), are largely attributed to its phenolic content, in particular to CGA. In addition, the content of CGA in eggplant increases after the thermal treatments normally used for eggplant cooking (Lo Scalzo *et al.*, 2010). Also, it is worth mentioning that, although some phenolic compounds are bitter (Macheix, 1990), bitterness present in some cultivars of eggplant is caused by saponins and glycoalkaloids (Aubert *et al.*, 1989; Sánchez-Mata *et al.*, 2010) and not by CGA, which does not cause appreciable bitterness at the concentrations present in eggplant (Nagel *et al.*, 1987). Therefore, breeding new cultivars of eggplant with enhanced CGA content is of interest, as these new cultivars would have a high added value derived from its improved nutraceutical properties without affecting its organoleptic properties.

4. Variation for CGA content in eggplant

Eggplant presents a wide morphological and molecular diversity (Hurtado *et al.*, 2012; Prohens *et al.*, 2005), as well as a broad variation for composition traits, including total phenolics and CGA content (Arivalagan *et al.*, 2012; Hanson *et al.*, 2006; Okmen *et al.*, 2009; Prohens *et al.*, 2007; Raigón *et al.*, 2008; Stommel and Whitaker, 2003). Few studies have been performed in which the variation for CGA content has been studied in a relevant number of eggplant accessions. The first and broadest study was performed by Stommel and Whitaker (2003), who found differences of up to 4.4-fold in the CGA content and a continuous range of variation in a collection of 97 accessions of cultivated eggplant from the core collection of the USDA-ARS collection. These same authors also studied seven commercial varieties and found differences in CGA content of up to 2.2-fold among them (Whitaker and Stommel, 2003). Another study was performed by Mennella *et al.* (2012), in which they studied the variation for CGA content in 10 accessions of eggplant at three ripening stages. These authors found differences of 2.8, 3.7, and 4.0-fold between accessions for the unripe, commercially mature, and physiologically ripe stages, respectively. Also, they found that the accessions of the non-Japanese type (containing the anthocyanin delphinidin-3-rutinoside in the fruit skin), on average, had a higher CGA content than the Japanese type (containing the anthocyanin nasunin in the fruit skin). However, a considerable diversity was found within each of these types (Mennella *et al.*, 2012). Overall, the results of these studies show that there is a wide diversity for CGA content within cultivated eggplant germplasm.

There are a few more studies in which, although CGA has not been measured, the total phenolics content has been estimated. In this respect, Hanson *et al.* (2006) found differences of up to 1.7-fold for total phenolics in a study involving 35 accessions of eggplant, Prohens *et al.* (2007) of up to 3.0-fold in a collection of 69 *S. melongena* accessions from different origins, Raigón *et al.* (2008) of up to 1.8-fold in a collection of 31 commercial varieties, landraces, and experimental hybrids, and Okmen *et al.* (2009) of 2.2-fold in a collection of 26 Turkish accessions of eggplant. A recent study, in which diversity for both CGA content and total phenolics were estimated (Mennella *et al.*, 2012) shows that variation for total phenolics in a collection of 10 eggplant accessions is lower than variation for CGA content. In this respect, these authors found differences of 2.4, 2.0, and 2.2-fold between accessions for the unripe, commercially mature, and physiologically ripe stages, respectively, which are lower values for relative differences than those observed for CGA content (see above). These results confirm the wide variation for phenolic content, and therefore for CGA content, in eggplant.

Non-genetic sources of variation can also contribute to the wide range of variation observed for CGA content in eggplant. Mennella *et al.* (2012) found important differences among fruit developmental stages. These authors found that there is a sharp decrease in CGA content during the fruit development, so that average values in a collection of 10 eggplant cultivars for the unripe, commercially mature, and physiologically ripe stages were of 21.6, 12.9, and 7.1 $mg{\cdot}kg^{-1}$, respectively. Also, important differences, which are nutritionally relevant, have been found by Whitaker and Stommel (2003) among different parts of the eggplant fruit. These authors found that the fruit flesh from midsection and blossom end part of the fruit had much higher content in CGA (on average 93% and 76% higher, respectively) than the stem end of the fruit. Also, Gajewski *et al.* (2009) found an average decrease of 37% in the CGA content after storage of eggplant for one week at 16°C. Contrarily, Concellón *et al.* (2012) found that storage for 14 days at 10ºC increased CGA content, while a reduction was observed when stored at 0°C.

Not much information exists for variation among years or cultivation conditions for CGA in eggplant. Mennella *et al.* (2010) found small (5%), although statistically significant, differences between two years for CGA content in eggplant genotypes; however, yearly differences were much higher (46%) for eggplant lines with introgressions from *S. aethiopicum* L. Hanson *et al.* (2006) also found very large and significant differences in total phenolics with average differences of 50% between two years. Regarding cultivation conditions, Luthria *et al.* (2010) did not find differences in CGA content when comparing eggplant fruits grown in two farms, one using conventional growing conditions and the other using organic cultivation. However, Raigón *et al.* (2010) found that in eggplants grown in the same farm, organically produced eggplants had 30% more total phenolic content than conventionally grown eggplants. An additional source of variation, in particular for comparing results from different research groups, comes from the methodology used for extraction and measurement of CGA (Luthria, 2012; Luthria and Mukhopadhyay, 2006).

All these data suggest that genetic, as well as many environmental factors (including extraction procedures), can affect the estimations of CGA content in eggplant and can contribute to differences observed among different works (Tab. 1). In particular, for an efficient breeding for CGA content it is important to include sufficient genetic diversity in the breeding programs as well as to reduce the non-genetic causes of variation and to standardize protocols for taking and processing samples.

5. Breeding strategies for increased CGA content in eggplant

Several strategies based on the exploitation of the naturally available variation can be applied for developing new cultivars of eggplant with increased chlorogenic content. A successful new commercial variety with improved concentrations of CGA will also require having good agronomic and commercial characteristics (i.e., good yield, lack of prickles, fruit shape and color adapted to consumer demands, etc.) (Daunay, 2008). Studies on variation for phenolic content, as well as new genomic information will be of great assistance for the development of these new improved cultivars.

The high intraspecific variation for CGA content and total phenolic content (Hanson *et al.*, 2006; Mennella *et al.*, 2012; Okmen *et al.*, 2009; Prohens *et al.*, 2007; Raigón *et al.*, 2008; Stommel and Whitaker, 2003; Whitaker and Stommel, 2003) can be used in several ways in conventional breeding programs. For example, selection among the accessions or varieties with highest CGA content can result in the identification of materials with higher content in CGA. However, very likely, landraces with high content in CGA will not present agronomic and commercial characteristics competitive with present modern varieties, and its practical utility as commercial varieties may be limited. An alternative is the development of hybrids between accessions or lines with high content in CGA and complementary for agronomic traits. Eggplant hybrids are known to be heterotic for yield (Rodriguez-Burrruezo *et al.*, 2008) and competitive with commercial hybrids in open field conditions (Muñoz-Falcón *et al.*, 2008). Prohens *et al.* (2007) and Raigón *et al.* (2008) studied the total phenolic content in eggplant landraces and hybrids among them. Some of these hybrids, in particular those involving one or both parents with high content in phenolics, had values close to those of the parent with the highest value. Also, these hybrids can be used, through several breeding methods (Acquaah, 2012), to select and develop inbred lines with higher content in CGA and improved agronomic and commercial characteristics or to introgress this trait in élite lines.

Cultivated eggplant can be hybridized, although with different degrees of success, with a group of related species, including wild species and the cultivated scarlet (*S. aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplants (Daunay, 2008). Some of these species have high contents in CGA, which could be introgressed into eggplant. For example, *S. incanum* presents high contents in CGA (Ma *et al.*, 2011; Prohens *et al.*, 2013; Stommel and Whitaker, 2003). *Solanum incanum* is considered as the putative ancestor of eggplant (Lester and Hasan, 1990) and interspecific hybrids and subsequent backcross generations to eggplant are fully fertile (Prohens *et al.*, 2013). The latter authors studied an interspecific family between *S. melongena* and *S. incanum* and found that even in the first backcross generation it was possible to select individuals with high content in CGA. This study also revealed that additive genetic effects were the most important in explaining CGA variation, suggesting that alleles from *S. incanum* should be placed in homozygous state to obtain a higher expression of the trait. Other species, like *S. sodomaeum* L. (=*S. linneanum* Hepper & Jaeger) also show a higher CGA content than that of *S. melongena* (Mennella *et al.*, 2010). However, eggplant lines with introgressions from *S. sodomaeum* did not present particularly high levels of CGA, very likely because these lines had not been selected for high CGA content (Mennella *et al.*, 2010).

Molecular breeding strategies can also be of great utility for developing eggplant cultivars with improved CGA content. The availability of genetic maps (Barchi *et al.*, 2010; Doganlar *et al.*, 2002; Fukuoka *et al.*, 2012; Wu *et al.*, 2009) can be useful for the detection of quantitative trait loci (QTLs) affecting CGA content, as has been done for other traits like anthocyanin content or parthenocarpy (Barchi *et al.*, 2012; Miyatake *et al.*, 2012). Also, the CGA synthesis pathway in Solanaceae (Fig. 2) is known (Clé *et al.*, 2008; Niggeweg *et al.*, 2004) and the sequences of the six genes codifying for the enzymes involved in this pathway (phenylalanine ammonia lyase, PAL; cinnamate 4-hydroxilase, C4H; 4-hydroxycinnamoyl-CoA ligase, 4CL; hydroxycinnamoyl-coA shilimate/quinate hydroxycinnamoil transferase, HCT; *p*-coumaroyl ester 3'-hydroxilase, C3'H; and, hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase, HQT) are available (Comino *et al.*, 2007, 2009; Joët *et al.*, 2010; Mahesh *et al.*, 2007; Menin *et al.*, 2010; Niggeweg *et al.*, 2004). In consequence, it is possible to map these genes and to study their co-segregation with QTLs for CGA content. Sequencing of these alleles in a collection of germplasm, as well as TILLING or EcoTILLING strategies, can be useful to identify allelic variants for these genes (Pérez-de-Castro *et al.*, 2012). A selection of the most favorable alleles for each of these, which could be pyramided in a single variety (Ishii and Yonezawa, 2007), could be done through analyses of gene expression, as has been done on coffee (Lepelley *et al.*, 2007).

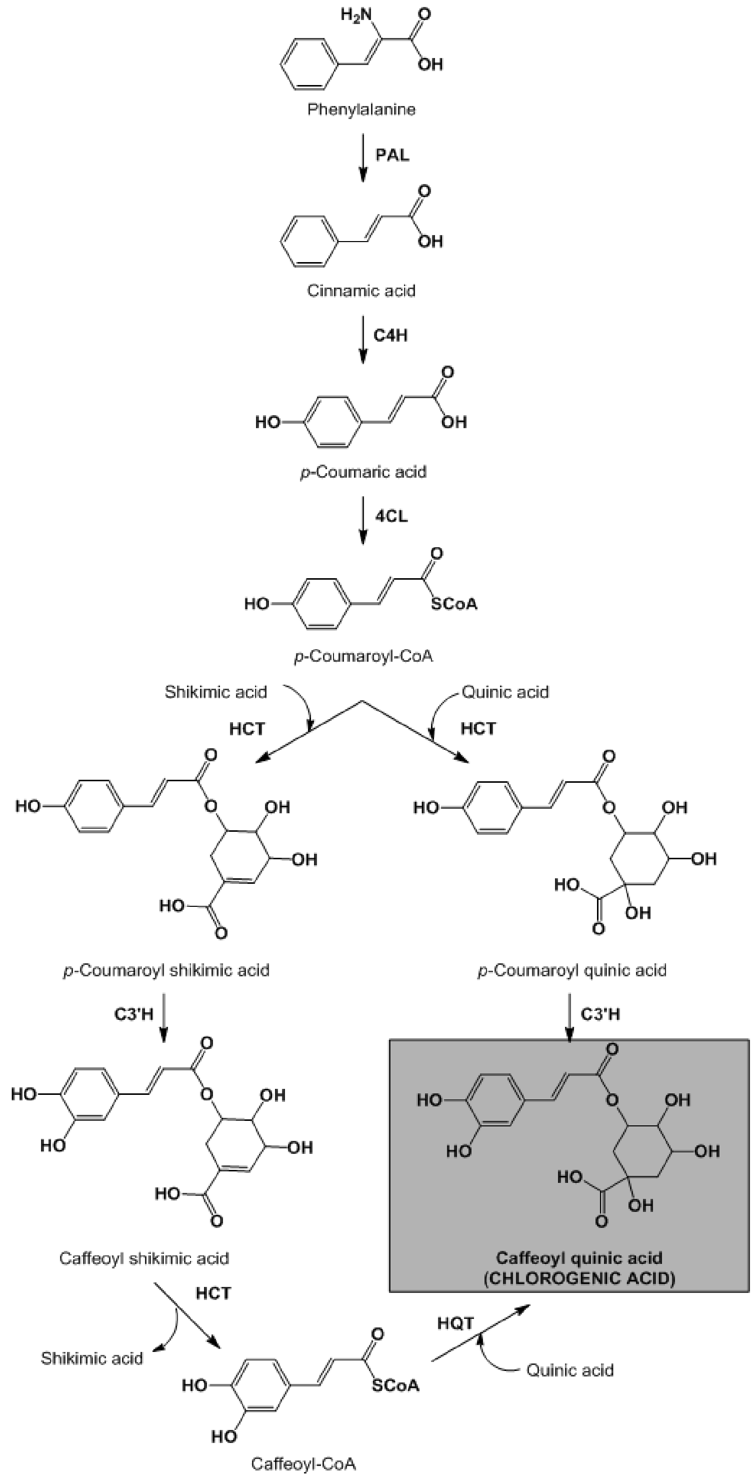

Fig. 2. Biochemical pathway for the synthesis of chlorogenic acid in eggplant (Clé *et al.*, 2008; Comino *et al.*, 2007, 2009; Joët *et al.*, 2010; Mahesh *et al.*, 2007; Menin *et al.*, 2010; Niggeweg *et al.*, 2004). Enzymes involved in the pathway are indicated: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxilase; 4CL, 4-hydroxycinnamoyl-CoA ligase; HCT, hydroxycinnamoyl-coA shilimate/quinate hydroxycinnamoil transferase; C3'H, *p*-coumaroyl ester 3'-hydroxilase; HQT, hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase

Genetic transformation has been successfully applied for several traits in eggplant (Acciarri *et al.*, 2000; Donzella *et al.*, 2000; Pal *et al.*, 2009). Niggeweg *et al.* (2004) obtained transgenic plants of tomato overexpressing the HQT enzyme, which resulted in accumulation of higher levels of CGA. This opens the way to use similar approaches in eggplant. However, many sectors from the society, especially in Europe, reject genetically modified (GM) plants and regulations for getting approval of GM cultivars are long, complicated, and expensive (Raybould and Poppy, 2012).

6. Fruit flesh browning as a side effect of CGA content improvement

As in other fruits and vegetables, like apple or artichoke, oxidation of phenolic compounds, including CGA, present in the eggplant flesh results in flesh browning, which reduces apparent quality (Adams and Brown, 2007; Macheix, 1990; Mishra *et al.*, 2012). When an eggplant fruit is cut open, the breakdown of the cellular compartments allows the orthophenolic compounds (hydroxycinnamic acid derivatives, like CGA) to be accessible to polyphenol oxidases (PPO), which catalyze their oxidation to quinones. Quinones then react non-enzymatically with O_2 and other molecules to produce compounds which cause the browning of the flesh in the cut area (Mishra *et al.*, 2012; Ramírez, 2002). Fujita and Tono (1988) found that CGA was the substrate for which eggplant PPO presented a greater affinity. However, Mishra *et al.* (2012) describe an intermediate specificity of eggplant PPO for CGA, showing only a 31% relative activity (using with 4-methylcatechol as 100% reference). In fact, eggplant PPO had a higher affinity for dihydrocaffeic acid or pyrocatachol, but a lower affinity for pyrogallol or gallic acid, than for CGA (Mishra *et al.*, 2012). In any case, under the same conditions, the higher the content in CGA in the fruit flesh, the higher the browning.

The fact that differences in PPO activity exist among eggplant cultivars (Dogan *et al.*, 2002; Mennella *et al.*, 2012) suggests that selection for low PPO activity could be carried out. In this way, simultaneous selection for low PPO activity and high content in CGA content could result in materials with greater functional quality and low browning. Also, other factors, like intracellular pH or ascorbic acid content, which affect the PPO activity (Concellón *et al.*, 2012; Doğan *et al.*, 2002; Mishra *et al.*, 2012), could play a role in the reduction of the degree of browning.

Prohens *et al.* (2007) studied the relationship between fruit flesh browning and the total phenolic content in eggplant. These authors found a wide variation for fruit browning among the cultivated germplasm, and a positive, but moderate, relationship between total phenolics content and fruit flesh browning (r=0.389), which indicates that it is possible to select varieties with high content in phenolics and low or moderate fruit flesh browning. More recently, the relationship between fruit flesh browning and total content in hydroxycinnamic conjugates (of which CGA was, by far, the most abundant) has been studied in an interspecific family between *S. melongena* and *S. incanum* (Prohens *et al.*, 2013). In this study it has been found that the correlation coefficient was low (r=0.245 for F2 and r=0.116 for the first backcross to *S. melongena*).

The fact that PPO genes in eggplant display considerable variation and that seem to be situated in a cluster in chromosome 8 (Shetty *et al.*, 2011) suggests that it is possible to use marker assisted selection for low PPO activity. Therefore, the data suggest that it is possible to select eggplant varieties with high content in CGA and low fruit flesh browning.

7. Conclusions

Given the many beneficial properties of CGA for human health and the high contents of this phenolic compound present in the eggplant fruit, developing new eggplant cultivars with improved functional quality resulting from increased CGA contents is of interest. The high genetic diversity among eggplant cultivars will facilitate the selection of sources of variation for high CGA content for breeding programs. Also, the use of wild relatives like *S. incanum* can result in the introgression of genes for high CGA values from these species. In both cases, the use of molecular breeding techniques, including marker assisted selection and the identification of allelic variants, can make an effective contribution to reaching this goal. Also, the low or moderate relationship between CGA content and fruit flesh browning together with selection for low PPO activity suggests that, in eggplant, it is possible to develop new cultivars with a combination of high CGA content and low fruit flesh browning.

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