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Characterization of Health-Related Compounds in Eggplant (*Solanum melongena* L.) Lines Derived from Introgression of Allied Species

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The purpose of the present study was to investigate the levels of either the nutraceutical and health-promoting compounds or the antioxidant properties of innovative eggplant (*Solanum melongena* L.) genotypes tolerant and/or resistant to fungi, derived from conventional and non-conventional breeding methodologies (i.e., sexual interspecific hybridization, interspecific protoplast electrofusion, androgenesis, and backcross cycles) in comparison with their allied and cultivated parents. Chemical measures of soluble refractometric residue (SRR), glycoalkaloids (solamargine and solasonine), chlorogenic acid (CA), delphinidin 3-rutinoside (D3R), total phenols (TP), polyphenoloxidase (PPO) activity, antiradical activity on superoxide anion and hydroxyl radical were carried out in raw fruit and peel of 57 eggplant advanced introgression lines (ILs), of three eggplant recurrent genotypes and of three allied species during 2005 and 2006. The majority of the ILs, obtained after several backcross cycles, showed positive characteristics with respect to the allied parents such as good levels of SRR, CA, D3R, TP, PPO activity, the scavenging activity against superoxide anion and hydroxyl radical and, in particular, significantly ($p \leq 0.05$) reduced concentrations of the toxic steroidal glycoalkaloids, solasonine and solamargine. These results showed the possibility to obtain new eggplant genotypes bearing useful traits derived from the allied parents (i.e., resistance/tolerance to plant pathogen fungi) together with nutraceutical and antioxidant properties typical of the cultivated species.

KEYWORDS: Eggplant breeding; disease resistance; solanaceous species; glycoalkaloids; polyphenols; antioxidants

INTRODUCTION

Genetic amelioration of crops, in the last decades, has been devoted to quality improvement of the products together with the introgression of disease resistances and other agronomical traits with increasing works aimed at exploiting natural genetic diversity including the allied germplasm (*1*). As far as the product quality is concerned, many studies on the healthy properties have been currently performed with regard to both the content in phytochemicals acting as antioxidants considered effective in the prevention of chronic diseases (*2*, *3*) and, especially for Solanaceae family, in the monitoring of the amount of antinutritional compounds (*4*, *5*). Hence, an agricultural production improved for both agronomical traits (i.e., yield and disease resistance) and healthy properties (i.e., low antinutritional factors and increased antioxidants contents) represents a main goal of the modern breeding programs.

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Eggplant (*Solanum melongena* L.) is one of the most cultivated vegetable crops world-wide, and it is ranked among the top six for the amount of its production (*6*). *Fusarium* and *Verticillium* spp fungal wilts, which cause high loss in production, are among its major cultivation problems (*7*). Recently, non-conventional and conventional (i.e., somatic and sexual hybridization) breeding methods enabled the introgression from allied species of tolerance and resistance traits to the diseases caused by *Verticillium dahliae* Kleb and *Fusarium oxysporum* f. sp. *melongenae*, respectively (*8*−*11*). The advanced introgression lines (ILs) derived from interspecific hybridization are very similar to their recurrent cultivated eggplant for almost all the phenotypical traits (*9*, *10*). However, the ILs might exhibit a variable level of some fruit compounds, such as glycoalkaloids and polyphenols, because fruit biochemical composition was never employed as selection criteria. To date, little information is available about the glycoalkaloids content in eggplant. Likewise, in other solanaceous species, glycoalkaloids may have the role to protect eggplant from attack by fungi, bacteria, insects, nematodes, slugs, etc. (*12−16*). Human toxicological studies showed that solamargine and solasonine, the major eggplant steroidal glycoalkaloids, when used at certain levels, showed toxic effects such as cell membrane disruption, acetylcholinesterase inhibition, liver damage, heart damage, teratogenicity, and embryotoxicity (*17−20*). However, it should also be mentioned that the cell disruption and the ability of these glycoalkaloids to bind with sterols and complex cholesterol may have beneficial effects if used in moderation. Thus, solamargine and solasonine have been used to inhibit cancer cells (*21−26*).

A remarkable diversity in polyphenol content and flesh browning was found in eggplant accessions and related species: Hanson et al. (*27*), found an average value of 952 mg/kg fw in *S. aethiopicum* and a range from 666 to 1265 mg/kg fw for eggplant accessions, while Prohens et al. (*28*) found an average value of 163 mg/kg fw in *S. aethiopicum* and a range from 280 to 830 mg/kg fw for eggplant accessions. Eggplant is particularly rich in nutraceutical compounds, such as polyphenols, that have already been characterized and measured: the main ones are chlorogenic acid (*28−30*) and the anthocyanin pigments delphinidin 3-rutinoside (D3R) and/or nasunin (*31−37*). It seems that polyphenols are strictly involved in antioxidant properties, due to their capacity in neutralizing free radical production (*27*, *38*).

The present paper deals with a study carried out on eggplant advanced introgression lines (ILs) derived from an interspecific sexual cross with *S. sodomaeum* or protoplast electrofusion with *S. aethiopicum* gr. *aculeatum* (= *S. integrifolium*) and gr. *gilo* (*10*). The fruits of these allied species showed a significantly different content of glycoalkaloids, phenols, and antioxidant profiles with respect to those of eggplant. Here we present the results of biochemical characterization and antioxidant properties of the fruits from ILs and parental genotypes (cultivated and allied species) for the above-mentioned traits performed over two subsequent years of the introgression breeding program.

MATERIALS AND METHODS

Plant Material. Two tetraploid somatic hybrids, 1F5(9)-Si [*S. melongena* line 1F5(9) (+) *S. integrifolium*; (*39*)] and D-Sa [*S. melongena* cv. Dourga (+) *S. aethiopicum*; (*40*)]; represented the initial materials. Anther culture was used to generate dihaploids progenies from the two interspecific hybrids (*41*, *42*). The androgenetic dihaploids born from anther culture of the tetraploid hybrid 1F5(9)-Si were successfully employed in the introgression breeding program of the resistance character to *Fusarium* into different breeding lines of eggplant, whereas for the D-Sa somatic hybrids, a first backcross at the tetraploid level between a 4× eggplant and the somatic hybrid was performed. These backcrossed plants were subjected to anther culture to obtain dihaploids which were successfully crossed with the recurrent eggplants (*43*).

The interspecific sexual hybrids with *S. sodomaeum* were obtained using the wild relative as the female parent. Backcrossing and selfing were carried out using different types of eggplant, including a line with a good level of *Verticillium* field tolerance (CCR3).

Commercially ripened fruits, visually evaluated according to the color of the skin and absence of mature seeds, were collected from field grown plants (18/plot) during 2005 and 2006 seasons in an experimental field located in Montanaso Lombardo (Lodi, Italy) at the Research Unit for Vegetable Crops of Agricultural Research Council of Italy (CRA). The genotypes employed were (a) the eggplant allied species *S. sodomaeum*, *S. aethiopicum*, and *S. integrifolium*; (b) the recurrent eggplant genotypes characterized by different shapes and colors of the fruit 1F5(9) (oval dark purple), Tal 1/1 (long dark purple) and CCR3 (long pale purple); (c) three groups of ILs, each derived from introgression breeding of the allied species (*10*) obtained by subsequent cycles of backcrosses (BC_{6-7}). In 2005, 35 ILs were analyzed (10 introgressed from *S. integrifolium*, 13 from *S. aethiopicum*, and 12 from *S. sodomaeum*). In 2006, 22 ILs were analyzed (6 introgressed from *S. integrifolium*, 13 from *S. aethiopicum*, and 3 from *S. sodomaeum*). The experimental sample was constituted by portions obtained from 5−8 fruits. Flesh cubes and peel slices of about 1.5−2 cm, made within 2 h after harvest, were immediately frozen in liquid N_2 and lyophilized. The freeze-dried tissue was powdered and held at −80 °C. All results were referred to as dry weight (dw).

Extractions and Analytical Conditions. The lyophilized and powdered eggplant tissue was treated in different ways for each performed assay.

Soluble refractometric residue (SRR) was measured on the centrifuged extract of 30 mg of eggplant powder with 1 mL of 1 mM HCl (5 min at room temperature), and it was expressed as percent substance on dw.

Glycoalkaloids, solamargine and solasonine, were extracted from 0.5 g samples of lyophilized fruit tissue by 95% ethanol as described by Birner (*44*) with some modifications. The analyses were performed by means of RP-HPLC according to Kuronen et al. (*45*), using partially purified solasonine and solamargine (kindly provided by Prof. Adelia Emilia de Almeida), as the external standard. The data were expressed as mg/100 g of dw (*46*, *47*).

Phenolic acids were extracted and analyzed according to Whitaker and Stommel (*48*) with minor modifications. The analyses were performed through a Waters E-Alliance HPLC system constituted by a 2695 separations module with quaternary pump, autosampler, and a 2996 photodiode array detector; data were acquired and analyzed with Waters Empower software on a PC. A binary mobile phase gradient of methanol in 0.01% acqueous phosphoric acid was used according to this procedure: 0−15 min, linear increase from 5 to 25% methanol; 15−28 min, linear increase from 25 to 50% methanol; 28−30 min, linear increase from 50 to 100% methanol; 30−32 min, 100% methanol; 32−36 min, linear decrease from 100 to 5% methanol; 36−43 min, 5% methanol. The flow rate was 0.8 mL/min. Quantification of chlorogenic acid (CA), carried out after a RP-HPLC separation, was based on absorbance at 325 nm relative to the sesamol internal standard and an external standard of authentic chlorogenic acid (Sigma-Aldrich, St. Louis, MO). The results were expressed as mmol/100 g of dw.

The fruits of the recurrent eggplant parents analyzed in the present study only had delphinidin-3-rutinoside (D3R) as peel anthocyanin, whereas the fruit peels of the allied parents did not exhibit detectable levels of D3R; hence, the ILs were exclusively analyzed for their D3R content. The extraction and the analysis of D3R were carried out on 200 mg of lyophilized and powdered peel, diluted in 10 mL of methanol containing 3% trifluoroacetic acid (TFA), as reported in Ichiyanagi et al. (*34*). RP-HPLC analysis was performed through the same Waters HPLC system as for CA analysis at a flow rate of 0.8 mL/min. Purified D3R (Polyphenols Laboratories AS, Sandnes, Norway) was used as the external standard in RP-HPLC separations. The results were expressed as mg/100 g of peel dw; the limit of detection was 1.3 mg/100 g of peel dw.

Total phenols (TP) and polyphenoloxidase (PPO) activity were assayed through spectrophotometric methods. TP was evaluated by a modified Folin-Ciocalteu method (*49*) on a McIlvaine buffer (pH 3.0, 1 mL) extract of 30 mg of lyophilized tissue. Results were expressed as mmol of CA per 100 g of dw. Chlorogenic acid was used because it is the main phenolic compound in the eggplant fruit.

The PPO activity was assayed following the Fujita and Tono (*50*) method, using 30 mg of lyophilized fruit extracted with 1 mL of McIlvaine buffer (pH 5.0). Results were expressed as U/100 mg of dw, with 1 U = 0.01 absorbance unit variation/min, using chlorogenic acid as the substrate at 420 nm (*30*, *51*).

The assays of antiradical activity were performed on superoxide anion and hydroxyl radical by electron spin resonance (ESR) spectrometry, following the method used by Privat et al. (*52*) and by Valavanidis et al (*53*), with some modifications. The free radical generation (2.8 mM KO_2-crown-ether-18-6 1:1 in dimethylsulfoxide for superoxide anion; 2 mM Fenton system in 0.1 M phosphate buffer pH 7.4 for hydroxyl radical) was followed by spin trapping with 5,5-dimethyl-1-pyrrolin-N-oxide 25 mM and 10 mM dissolved in 0.1 M phosphate buffer pH 7.4 for superoxide anion and hydroxyl radical, respectively. The reaction was elapsed for exactly 1 min, after this time the ESR spectra were recorded in the presence and absence of eggplant extract (supernatant of 30 mg of eggplant powder with 1 mL of HCl 1 mM), respectively. In order to calculate the scavenging index, the main band amplitude measure was used applying this equation: $I = 100 - (I_x/I_0 \times 100)$, where I_x is the spectrum amplitude in the presence of eggplant extract and I_0 is the spectrum amplitude in its absence.

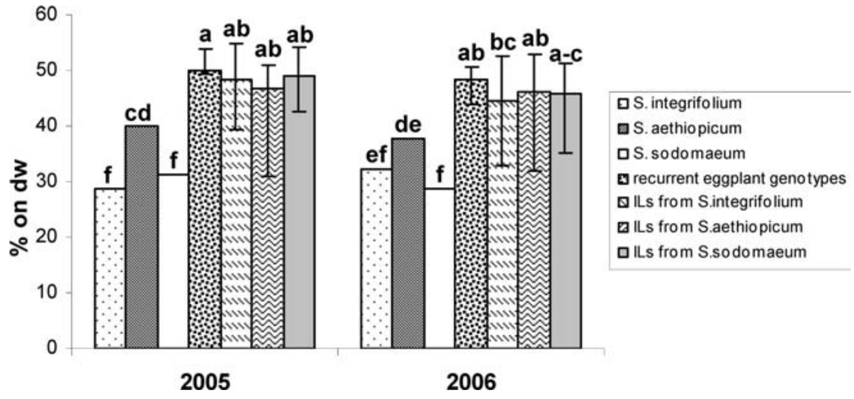

Figure 1. Soluble refractometric residue (SRR, % dw) in fruits of the three *Solanum* spp., the pools of the recurrent eggplant genotypes and ILs analyzed. Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). Bars indicate the min−max values within each pooled genotype.

The results were expressed as mmol of CA per 100 g of dw, by interpolating the data from eggplant extracts with the scavenging index of chlorogenic acid solutions at known concentrations.

Statistical Data Treatment. For each year, all the determinations were carried out at least three times. The statistical analyses here presented were performed using the pooled average values of the ILs introgressed from the same donor species, the average value of the three recurrent eggplant genotypes, and the values of the allied donor species. Data were subjected to ANOVA according to a completely randomized design. Means were compared by using Tukey HSD test ($p \leq 0.05$). To show a better picture of the data gathered, the maximum and minimum values within the recurrent eggplant genotypes and the ILs have been also shown.

Moreover, ANOVA statistical analyses and Tukey HSD test were performed separately for each year using the data of the single genotypes employed (the results of this statistical analysis are presented as supporting online information). The correlation indexes (rxy) were measured by simple linear regression analysis.

RESULTS AND DISCUSSION

The SRR (**Figure 1**), representing all main soluble compounds, showed a similar pattern in both sampling years. Highly significant differences were detected among the genotypes tested ($P < 0.0001$). A significant difference ($P < 0.0263$) was also evidenced between the two years. However, the recurrent eggplant genotypes and the allied species gave similar results in the two sampling years. A low SRR percentage was shown in the allied species with respect to the pools of the recurrent eggplants and the ILs. Among the allied species, *S. aethiopicum* showed a higher value with respect to the other two species. The ILs showed a large variation exhibiting values similar either to the donor allied genotypes or to the recurrent eggplants (see Supporting Information).

The allied species evidenced solasonine values significantly higher than that of the average content in the ILs and recurrent eggplants (**Table 1**). The eight pools of ILs and recurrent eggplants, and *S. integrifolium* did not show any significant difference in the two sampling years, whereas, between the two years, a significant variation was detected in the solasonine content of *S. aethiopicum* (2005 > 2006) and *S. sodomaeum* (2005 < 2006). The highest glycoalkaloid levels were detected in *S. sodomaeum* (610.50 and 690.51 mg/100 g of dw in 2005 and 2006, respectively). In each year, one single IL introgressed from *S. sodomaeum* had a solasonine content statistically not different (line 182 in 2005 and 352 in 2006 with 59.14 and 85.83 mg/100 g of dw, respectively) from that of *S. integrifolium*, while the remainder of the ILs showed values similar or even lower than those detected in the recurrent eggplants (see Supporting Information).

A similar behavior was observed for solamargine contents (**Table 1**), as demonstrated by the highest correlation index found between the two eggplant glycoalkaloids (rxy = 0.80). The allied species (except *S. aethiopicum* in 2006) had significantly higher amounts with respect to both the ILs and recurrent eggplant pools. Only *S. aethiopicum* and *S. sodomaeum* had a significantly higher amount of solamargine in the 2005 with respect to 2006. In particular, *S. sodomaeum* showed a 4-fold increment (769.57 vs 185.04 g of dw, respectively) having the highest content in both years. Yet, one single introgressed line from *S. sodomaeum* in 2005 (line 182 with 92.31 mg/100 g of dw) and three ones from *S. integrifolium*, *S. aethiopicum*, and *S. sodomaeum* in 2006 (lines 494, 352, 167 with 99.62, 84.61, and 54.74 mg/100 g of dw, respectively) had a solamargine content similar to or higher than that of *S. integrifolium* and *S. aethiopicum* (see Supporting Information).

The variation in the glycoalkaloids content between the two sampling years mainly regarded the allied species, particularly *S. sodomaeum*, with respect to the recurrent eggplants. Probably, as reported in potato (*54*) and tomato (*55*), genetic as well as environmental effects may cause such variation. Solasonine and solamargine total amount in allied species was close to or exceeded the recommended safety value. Several authors suggest, as for potato glycoalkaloids in tuber, that the level of total glycoalkaloids should not exceed 200 mg/kg of fw (or 200 mg/100 g of dw) (*46*, *47*). In the fruits of *S. integrifolium*, *S. aethiopicum*, and *S. sodomaeum*, the total content in the two sampling years averaged 173.63, 210.87, and 1127.80 mg/100 g of dw, respectively, whereas in the fruits of recurrent genotypes and of ILs introgressed from *S. integrifolium*, *S. aethiopicum*, and *S. sodomaeum* such contents averaged 27.54, 37.51, 19.02, and 54.66 mg/100 g of dw, respectively. These results point out that in the genetic improvement of eggplant through introgression breeding from its related species, it is important not to drag unsafe features, such as a high level of toxic compounds, together with the useful trait(s). The need to perform biochemical analyses is crucial, as in the present breeding program, when the allied parents possess high glycoalkaloid content in their fruits. Biochemical analyses performed in the last cycles of the breeding program were effective to recover the majority of the ILs with glycoalkaloid content similar to that of the recurrent eggplant genotypes. Therefore, the backcross cycles successfully reduced solamargine and solasonine levels in ILs also in the absence of selection for low glycoalkaloid content.

The content of chlorogenic acid, the major monomeric phenolic compound in eggplant fruits, is shown in **Table 1**. In both sampling years, CA was significantly higher in both the pools of ILs and recurrent eggplants, and *S. sodomaeum* with respect to the allied species *S. integrifolium* and *S. aethiopicum*. CA contents in *S. sodomaeum* were the highest ones (6.22 and 9.22 mmol/100 g of in 2005 and 2006, respectively); the other two allied species showed the lowest values (the average being less than

Table 1. Solasonin, Solamargin, Chlorogenic Acid (CA), Delphinidin 3-Rutinoside (D3R), and Total Phenols (TP) Content in Fruits of the Three *Solanum* spp., the Pools of the Recurrent Eggplant Genotypes, and ILs Analyzed[a]

genotype	solasonine[b] (mg/100 g of dw)	solamargine[b] (mg/100 g of dw)	CA[b] (mmol/100 g of dw)	D3R[b] (mg/100 g of peel dw)	TP[b] (mmol of CA eq/100 g of dw)
			2005		
S. integrifolium	68.1 de	80.9 cd	1.28 f	<1.3[c] i	1.64 d
S. aethiopicum	178.5 c	107.2 c	0.59 g	<1.3[c] i	2.09 d
S. sodomaeum	610.5 b	769.6 a	6.22 b	<1.3[c] i	12.83 a
recurrent eggplant genotypes	10.9 (9.2−12.5) f	26.7 (22.9−29.9) e	4.37 (3.16−6.76) c	200.5(81.6−406.7) e	5.45 (4.40−7.42) bc
ILs from *S. integrifolium*	11.0 (1.9−33.7) f	15.3 (5.1−31.2) e	2.29 (1.62−3.91) e	36.6 (<1.3[c]-88.7) g	5.13 (3.75−6.93) bc
ILs from *S. aethiopicum*	6.2 (2.7−10.2) f	12.5 (2.6−23.1) e	2.28 (0.83−2.94) e	65.4 (<1.3[c]-295.2) f	4.52 (3.10−6.24) c
ILs from *S. sodomaeum*	9.6 (1.7−59.1) f	29.2 (7.3−92.3) e	3.60 (1.94−5.78) d	20.6 (<1.3[c]-62.9) h	6.21 (4.27−9.10) bc
			2006		
S. integrifolium	96.2 d	102.1 c	0.22 g	<1.3[c] i	2.11 d
S. aethiopicum	91.3 d	44.8 de	0.45 g	<1.3[c] i	1.90 d
S. sodomaeum	690.5 a	185.0 b	9.22 a	<1.3[c] i	11.86 a
recurrent eggplant genotypes	3.6 (0.0−10.7) f	13.9 (0.0−23.4) e	4.58 (3.87−5.92) c	555.7(394.2−811.8) a	6.43 (5.56−7.90) bc
ILs from *S. integrifolium*	15.3 (0.0−65.7) ef	30.3 (3.3−99.6) e	2.20 (0.52−3.07) e	269.7(1.8−474) d	5.50 (4.47−5.93) bc
ILs from *S. aethiopicum*	8.1 (0.0−26.4) f	14.0 (0.0−54.7) e	3.32 (1.19−5.07) d	295.8(1.8−568.7) c	5.58 (3.06−7.16) bc
ILs from *S. sodomaeum*	35.2 (0.0−85.8) ef	35.1 (7.3−84.6) e	4.84 (2.42−6.34) c	424.9(212.6−752.8) b	6.68 (5.51−6.94) b

[a] Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). [b] Mean values (concentration range). [c] Limit of detection.

1.0 mmol/100 g of dw). The average content in CA of all eggplant genotypes in both years (about 3.43 mmol/100 g of dw, range 0.52−6.76) resulted in full accordance with the data of Whitaker and Stommel (*48*). The amount of CA differed significantly in the two years. Except ILs pool derived from *S. sodomaeum* in 2006, the other ILs pools showed CA values significantly lower than the cultivated pools. The 6−7 successive backcrosses with the recurrent parents, probably, contributed either in increasing the levels of CA in ILs from *S. integrifolium* and *S. aethiopicum* or in decreasing the levels in ILs from *S. sodomaeum* when compared to the corresponding allied parents.

Fruit of recurrent eggplant and ILs pools always showed higher D3R levels in 2006 with respect to 2005 (**Table 1**). This fact can be interpreted by an environmental variability, because the anthocyanin level can be strongly influenced by some environmental conditions, such as a difference in temperature (*56*). The allied species did not show detectable levels of the anthocyanin. In both years, the average D3R content in the recurrent eggplants was significantly higher than those of ILs. However, the large variability present in the introgressed lines enabled one to identify single introgressed lines, regardless of the donor allied species, bearing pigmented fruits exhibiting D3R content similar to those either of the recurrent genotypes or, only in 2005, of allied species (see Supporting Information).

The TP (**Table 1**) of allied species showed the highest values in *S. sodomaeum* (the two years averaged about 12 mmol of CA eq/100 g of dw), while *S. integrifolium* and *S. aethiopicum* showed lower values, about 2 mmol of CA eq/100 g of dw, resembling the trend shown by chlorogenic acid, with a significant correlation index (rxy = 0.78). The average of TP content in recurrent eggplant genotypes was close to 5.9 in both years (5.5 and 6.4 mmol of CA eq/100 g of dw, in 2005 and 2006, respectively), a TP value in full accordance with other studies, which ranged from 4.7 to 5.9 mmol of CA eq/100 g of dw (*30*), and only partially with refs *27* and *28*, where our data are about double that found by other authors. The average TP values in the three ILs were similar to each other and not statistically different from the recurrent genotypes. No great variability, when compared to other evaluated traits, was found among the lines of the same ILs pool (see Supporting Information).

Taking into account the TP, CA, and D3R content of the ILs, particularly in the 2006 ones, it can be noted that some advanced ILs simultaneously displayed a level of these health-related compounds not statistically different from those of the original parental genotypes (recurrent eggplants or allied species) which had the highest value. This is the case, for example, of the IL 363 which had both CA and TP contents not different from the allied donor *S. sodomaeum* and a D3R content similar to that of the recurrent eggplants; a comparable behavior was shown by the ILs 238 and 214 introgressed from *S. aethiopicum* and the IL 187 introgressed from *S. integrifolium* (see Supporting Information). These data demonstrate that lines carrying useful biochemical features coming from the different species involved in the breeding program can be found, although the selection was made for fungal wilts resistance and morphological traits.

The PPO activity indicates the tendency to browning of eggplant flesh, and it is commonly considered an important quality index for this produce: a low PPO activity is a positive trait because leads to a decrease in flesh browning when the fruit is cut. It generally shows a higher variability with respect to other measured traits, because of the influence of different factors, for example, polyphenol quality and quantity, intrinsic enzyme activity, and acidity of the medium (*57*): in fact, PPO data showed no significant correlation with other biochemical traits. In our study, the PPO profile exactly followed the same trend in the two sampling years (**Figure 2**). A very low activity was found in the allied species, especially in *S. sodomaeum*. The highest PPO activity was detected in the recurrent genotypes, and it was significantly higher with respect to either the allied species (average value about 9-fold) or ILs (average value 2-fold). However, a very large variability was detected among the lines belonging to the recurrent and ILs pools (see Supporting Information).

Superoxide scavenging data of two years (**Figure 3**) were positively correlated with TP (rxy = 0.42), reaching a higher value in 2006 (rxy = 0.71); these results are in accordance with those reported by Hanson et al. (*27*). In both sampling years, *S. sodomaeum* and the pool of recurrent eggplant had significantly higher superoxide anion scavenging capacity than the other two allied species (about 1.2 mmol of CA eq/100 g of dw), which showed the lowest values. In 2006, all the ILs values were close to that of recurrent eggplants, with a relatively low min−max variability with respect to those found in 2005. In 2005, a low value was found in ILs from *S. integrifolium*, and an intermediate

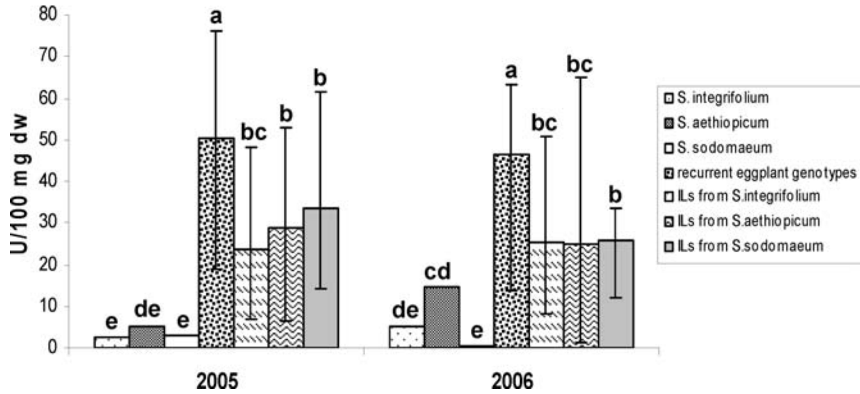

Figure 2. Polyphenoloxidase (PPO, U/100 mg of dw) activity in fruits of the three *Solanum* spp., the pools of the recurrent eggplant genotypes and ILs analyzed. Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). Bars indicate the min−max values within each pooled genotypes.

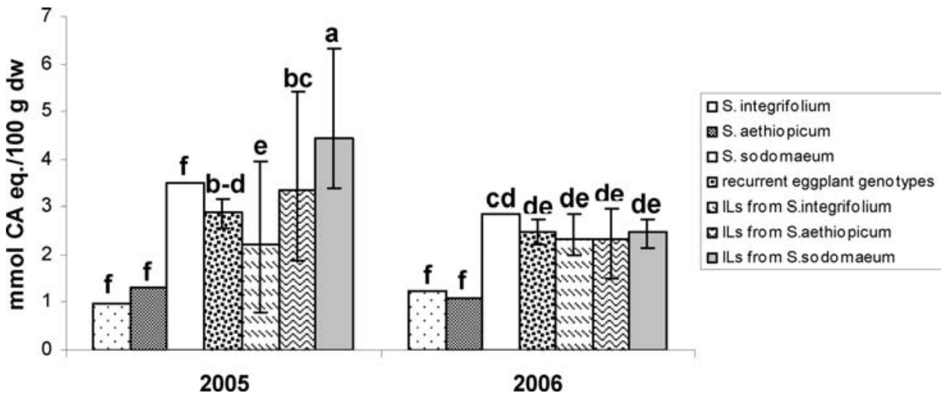

Figure 3. Superoxide anion scavenging (mmol of CA eq/100 g of dw) capacity in fruits of the three *Solanum* spp., the pools of the recurrent eggplant genotypes and ILs analyzed. Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). Bars indicate the min−max values within each pooled genotype.

one in the ILs from *S. aethiopicum*. The highest average value (about 4.4 mmol of CA eq/100 g of dw) was found in the ILs from *S. sodomaeum*, exceeding in 9 out of 12 ILs that of its allied parent (see Supporting Information). This may suggest that in the ILs the backcrosses cycles enable values of activity against superoxide anion similar to those of the recurrent eggplants and *S. sodomaeum*. The trend of antioxidant capacity against superoxide anion, especially in samples from ILs, was only partially in agreement with TP and the content of CA and D3R. This could support the possibility that other compounds could be involved in the scavenging capacity against such an anion.

The scavenging measures against the hydroxyl radical (**Figure 4**) are much more higher (total average 27.1 mmol of CA eq/100 g of dw) than those against superoxide anion (total average 2.9 mmol of CA eq/100 g of dw). This is due to the very fast reaction occurring when hydroxyl radical is involved and, consequently, a higher amount of scavenger is needed. The total average for 2005 (22.0 mmol of CA eq/100 g of dw) was significantly different from 2006 (34.6 mmol of CA eq/100 g of dw), probably because of a strong seasonal effect on the scavenging potential of eggplant samples. This difference between the two years reflected that of anthocyanins levels, in fact a positive linear correlation (rxy = 0.55) of scavenging of hydroxyl radical was only found with the D3R amounts. This is in accordance with previous works that demonstrated the specific effect of eggplant's pigments in the scavenging of hydroxyl radical (*32*, *33*).

In allied genotypes, the lack of D3R and the strong differences, found between the two years, in the scavenging activity toward hydroxyl radical (**Figure 4**) could suggest the involvement of other substances and/or of environmental influence. Such hypothesis is enhanced by the finding that the fruit extracts from *Solanum nigrum* and *Solanum torvum*, two eggplant allied species, containing nonanthocyanic compounds, were revealed to be effective scavengers of hydroxyl radicals (*58*, *59*).

Moreover, in the 2005 samples, the hydroxyl radical activity of ILs was not different from that of the recurrent eggplants, whereas in 2006 samples, the ILs from *S. integrifolium* and *S. sodomaeum* showed significantly lower activity.

In conclusion, our study demonstrates that most of the new eggplant ILs obtained from introgression breeding from three allied species, on average, generally displayed a biochemical composition and functional properties similar to that of the recurrent eggplants employed in the 6−7 cycles of backcrosses. During the introgression-breeding program, selection was exclusively applied for resistance to *Fusarium* and tolerance to *Verticillium*, and for phenotypic features. The lines here analyzed carry the *Fusarium* resistance locus *Rfo-sa1* from *S. aethiopicum* or *S. integrifolium* and tolerance to *Verticillium* wilt from *S. sodomaeum*; most of them are fertile, vigorous, productive, and display fruit shape, size, and color similar to the most common Italian cultivars; therefore, these lines represent valuable putative parents for the synthesis of commercial F1 hybrids (*9*, *10*).

The biochemical survey performed at the end of the backcross cycles was particularly important for the glycoalkaloid content. Even though the allied species employed have a high content of solamargine and solasonine, the ILs had total glycoalkaloids content well below the recommended safety value given for potatoes (200 mg/100 g of dw), although a certain degree of variability was evidenced among the introgressed lines. The other

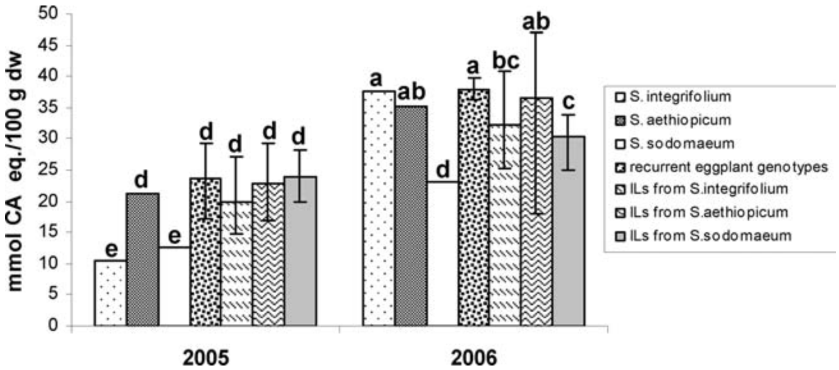

Figure 4. Hydroxyl radical scavenging capacity (mmol of CA eq/100 g of dw) in fruits of the three *Solanum* spp., the pools of the recurrent eggplant genotypes and ILs analyzed. Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). Bars indicate the min-max values within each pooled genotypes.

quality traits (SRR, CA, D3R, PPO, TP, superoxide anion, and hydroxyl radical scavenging) analyzed in the ILs displayed, similarly to glycoalkaloids, a variability that can allow selection of ILs enhanced for qualitative traits (e.g., TP content or reduced PPO activity). However, no clear link can be now gathered between the variation in the glycoalkaloids, phenols profile and antioxidant activity and the enhanced response to fungal wilts observed in the improved ILs. Further molecular and biochemical dissection of the traits studied may confirm whether the emerged variation is associated with the introgression into the eggplant genome, of other exotic genes from the allied species in addition to the resistance/tolerance ones.

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Note Added after ASAP Publication

There were changes made to the caption and footnote *a* of Table 1 in the version of this paper published ASAP on June 9, 2010; the correct version published on June 11, 2010.

Supporting Information Available: ANOVA statistical analyses and Tukey test for each year using the data of the single genotypes employed. This material is available free of charge via the Internet at http://pubs.acs.org.

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