Secondary Metabolite Profile in Induced Tetraploids of Wild *Solanum commersonii* Dun.

by **Immacolata Caruso**[a])[1]), **Laura Lepore**[b])[1]), **Nunziatina De Tommasi**[b]), **Fabrizio Dal Piaz**[b]), **Luigi Frusciante**[a]), **Riccardo Aversano**[a]), **Raffaele Garramone**[a]), and **Domenico Carputo***[a])

[a]) Department of Soil, Plant, Environmental, and Animal Production Sciences (DISSPAPA), University of Naples Federico II, Via Università 100, I-80055 Portici (Na) (phone: +39-081-2539225; fax: +39-081-2539481; e-mail: carputo@unina.it)

[b]) Department of Pharmaceutical Sciences, University of Salerno, Via Ponte don Melillo, I-84084 Fisciano (SA)

The main aim of this work was to study the leaf secondary metabolite profiles of artificially induced tetraploids ($2n=4x=48$) of *Solanum commersonii*, a diploid ($2n=2x=24$) wild potato species. The tetraploid genotypes of *S. commersonii* were produced by oryzalin treatment. Both HPLC-UV and LC/MS analyses revealed that there were no qualitative differences in the metabolite profiles between the diploid *S. commersonii* and its tetraploids. By contrast, the results showed that the phenylpropanoid content was generally significantly higher in the tetraploids than in the diploid *S. commersonii*. Concerning the glycoalkaloids (GAs), the results provided evidence that the content of minor GAs (solanidenediol triose, solanidadienol lycotetraose, and solanidenol lycotetraose) was higher in tetraploids than in the diploid progenitor, while the content of major GAs (dehydrodemissine and dehydrocommersonine) was significantly higher in diploid *S. commersonii* than in its tetraploid genotypes. The results are discussed from the practical perspective of potato biodiversity enhancement.

Introduction. – The rule that somatic nuclei contain two complete sets of chromosomes is subjected to several exceptions in the plant kingdom, where species possessing more than two full sets of chromosomes are often present. This phenomenon has been referred to as polyploidy. A triploid has three sets of chromosomes, a tetraploid four, a pentaploid five, *etc*. Polyploidy has a fundamental role in the speciation, biodiversity dynamics, and adaptability of several angiosperms, including important crops such as alfalfa, potato, cassava, banana, apple, and sugar beet [1][2]. It is often associated with novel phenotypes that are not present in diploid progenitors [3–5], possibly extending habitats in which normal development can take place. Polyploid plants often show an increase in leaf, flower, fruit, organ, and cell size, in biomass [6–8], in gene activity, and in enzyme diversity [9]. Moreover, they often present late and longer flowering [10][11] and higher tolerance to environmental stresses [12][13]. Polyploidy can also affect secondary metabolites both in terms of quantities and chemical diversity. All these effects result from the fact that a polyploid genome is not only the strict sum of the progenitor genomes, but it is a new combination due to structural and functional modifications induced by polyploidization events.

[1]) These authors contributed equally to this work.

Indeed, duplicated genes may be lost, maintained, or diverged, assuming new functions and, thus, providing new phenotypes.

Polyploidization can be artificially induced to increase the production and/or improve the quality of important medicinal compounds, such as pharmaceuticals, and aroma chemicals [14]. Polyploid *Artemisia annua*, *Chamomilla recutita*, *Salvia miltiorrhiza*, and *Scutellaria baicalensis* produced more flavonoids or terpenoids per gram of tissue than their diploid counterparts [15–18]. Moreover, the results obtained by *Kim et al.* [19] demonstrated the benefit of polyploidy induction on the ginsenoside accumulation in adventitious roots of ginseng. Synthetic polyploids may also show an altered profile of secondary metabolites. In tetraploids of *Mentha spicata*, *Dhawan* and *Lavania* [14] reported a lower concentration per unit dry weight of essential oils than in the diploid genotypes. Since the mechanisms underlying these phenomena are still poorly understood, studies with artificially induced polyploids may contribute to improve the knowledge of the effects of gene dosage on secondary-metabolite pathways and explain apparent contradictions. In addition, due to the fact that polyploidization is often used in plant breeding to exploit biodiversity and overcome sexual barriers [20], the knowledge of the characteristics of newly generated polyploids is crucial for the efficient selection of plant materials.

Within the framework of a potato-breeding program exploiting resistance genes possessed by diploid ($2n=2x=24$) incongruent *Solanum commersonii*, we produced synthetic tetraploids ($2n=4x=48$) of this wild species. As there is only scant information on the effect of polyploidization in *Solanum*, in this study, the leaf phenylpropanoid and glycoalkaloid (GA) contents between artificially induced tetraploids of *S. commersonii* and the diploid progenitor were compared. Our research was focused on the leaves, since polyploidy may induce metabolic changes that result in stronger plant defense systems.

Results and Discussion. – *Polyploidy Induction and Verification.* The effect of oryzalin on the survival of shoot tips was assessed about three weeks after the treatment of the diploid *S. commersonii* clone (named *cmm 1T*), and the results indicated that the explant mortality was only 18% (data not shown). After chloroplast and chromosome counting, four plants (coded *cmm pt 15*, *cmm pt 23*, *cmm pt 24*, and *cmm pt 30*) were selected as tetraploid test plants. The results confirmed that *in vitro* oryzalin treatment of non-flowering shoot tips represented a good alternative to chromosome doubling by *in vitro* tissue culture, avoiding problematic genetic variation. Compared to the use of colchicine, the advantage of oryzalin is the higher binding activity in plant tubulins and, hence, the higher microtubule depolymerizing activity [21].

Analysis of S. commersonii *Leaf Extracts.* The leaf extracts of the *Clone cmm 1T* of *S. commersonii* were analyzed using an HPLC system equipped with an *Onyx* C_{18} monolithic column and coupled with an ESI-Q-TOF mass spectrometer or with a diode array detector (*Fig.*). Twenty compounds were identified [22] and quantified (*Table 1*). The peak assignments were achieved on the basis of UV and MS spectra, and by comparison of their chromatographic properties with those of pure standards, when available. Chlorogenic acid (3-*O*-caffeoylquinic acid, 3-CQA) was by far the most abundant phenylpropanoid. An isomer of 3-CQA, neochlorogenic acid (5-*O*-caffeoylquinic acid, S-CQA) was also identified, and it was present at 7.4% of 3-CQA. In *Clone*

cmm 1T, 3-CQA was present at 60.19 mg/100 g dry tissue (*Table 1*) and constituted over 65% of the total soluble phenylpropanoid derivatives. Caffeoylquinic acid, caffeic acid, ferulic acid, ferulic acid amide, and 4,5-di-*O*-caffeoylquinic acid were also detected. Among the compounds with a caffeoyl component, three dihydrocaffeoyl polyamines were identified on the basis of their UV and mass spectra, *i.e.*, tris(dihydrocaffeoyl)-spermine, 4,5-di-*O*-caffeoylbis(dihydrocaffeoyl)spermine, and bis(dihydrocaffeoyl)-spermidine. Moreover, seven GAs were also identified.

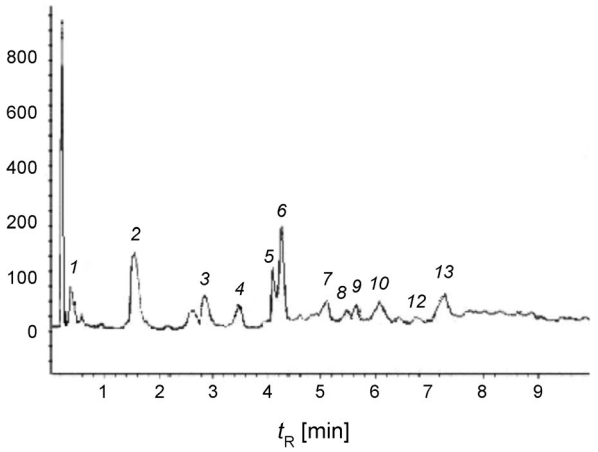

Figure. *HPLC Chromatogram of the MeOH extract of* S. commersonii *leaves. 1*, Ascorbic acid; *2*, tryptophan; *3*, caffeoylquinic acid; *4*, caffeic acid; *5*, neochlorogenic acid (5-CQA); *6*, chlorogenic acid (3-CQA); *7*, bis(dihydrocaffeoyl)spermine; *8*, bis(dihydrocaffeoyl)spermidine; *9*, ferulic acid; *10*, ferulic acid amide; *12*, 4,5-di-*O*-caffeoylquinic acid; *13*, rutin.

Glycoalkaloid Identification. Few GA standards are commercially available and most of them are aglycones. Consequently, we determined the structure of the GAs by LC/ESI-MS, according to the *m*/*z* values of protonated molecular ions ($[M+H]^+$) and to the fragment ions detected in the MS^2 experiments. Our results were then compared to those reported in the literature [22–24]. All GAs found in the *cmm 1T* leaf extracts belong to the solanidane family, as suggested by the presence in most of the acquired MS^2 spectra of the product ions with peaks at *m*/*z* 271 and 253, resulting from an *E*-ring rearrangement [23]. Dehydrocommersonine and dehydrodemissine were the most abundant GAs identified. The former was eluted at 8.52 min and showed the peak of the $[M+H]^+$ ion at *m*/*z* 1046.57, indicating a molecular formula of $C_{51}H_{83}NO_{21}$ (calc. 1046.55). Fragmentation of the $[M+H]^+$ ion produced ions with peaks at *m*/*z* 884.52 ($[M-Glu+H]^+$), 722.49 ($[M-2\,Glu+H]^+$), 560.40 ($[M-3\,Glu+H]^+$), 398.31 ($[M-3\,Glu-Gal+H]^+$), and 380.33 ($[M-3\,Glu-Gal-H_2O+H]^+$; *Table 2*). These data confirmed commertetraose as carbohydrate moiety and solanidine as aglycone (fragment ion with peak at *m*/*z* 398.30), as inferred by the presence of the product ions with peaks at *m*/*z* 271 and 253 [22][24]. Dehydrodemissine was eluted at 8.7 min, showing the peak of the $[M+H]^+$ ion at *m*/*z* 1016.56, suggesting a molecular formula of $C_{50}H_{81}NO_{20}$ (calc. 1016.54). MS^2 Analysis (*Table 2*) revealed the presence of

Table 1. *Secondary Metabolite Content of* Solanum commersonii *Leaves of the Diploid Progenitor* cmm 1T *and Its Tetraploid Derivatives* cmm pt 15, cmm pt 23, cmm pt 24, *and* cmm pt 30

Secondary metabolite	Content [mg/100 g of dried tissue][a])				
	cmm 1T	*cmm pt 15*	*cmm pt 23*	*cmm pt 24*	*cmm pt 30*
Phenylpropanoids					
Caffeoylquinic acid	2.88 ± 0.08 (a)[b])	4.79 ± 0.05 (b)	6.78 ± 0.03 (c)	7.33 ± 0.75 (c)	7.24 ± 0.57 (c)
Caffeic acid	7.63 ± 0.21 (a)	9.90 ± 0.19 (b)	10.38 ± 0.04 (c)	15.17 ± 0.06 (d)	10.12 ± 0.16 (b)
Neochlorogenic acid (5-CQA)	4.50 ± 0.43 (b)	4.40 ± 0.23 (b)	5.51 ± 0.02 (c)	6.70 ± 0.07 (d)	1.47 ± 0.13 (a)
Chlorogenic acid (3-CQA)	60.19 ± 0.69 (d)	55.68 ± 0.30 (c)	45.41 ± 0.17 (a)	66.72 ± 0.46 (e)	48.94 ± 0.37 (b)
Bis(dihydrocaffeoyl)-spermine	4.29 ± 0.15 (a)	5.64 ± 0.44 (c)	4.81 ± 0.04 (b)	6.48 ± 0.07 (b)	7.05 ± 0.08 (e)
Bis(dihydrocaffeoyl)-spermidine	8.88 ± 0.32 (b)	4.21 ± 0.07 (a)	12.28 ± 0.03 (d)	16.14 ± 0.15 (e)	11.17 ± 0.10 (c)
Ferulic acid	9.52 ± 0.35 (b)	13.11 ± 0.38 (d)	11.32 ± 0.01 (c)	17.59 ± 0.13 (e)	7.65 ± 0.08 (a)
Ferulic acid amide	6.71 ± 0.60 (b)	7.91 ± 0.49 (b)	11.17 ± 0.03 (c)	13.61 ± 1.79 (d)	N.D.[c])
Tris(dihydrocaffeoyl)-spermine	7.90 ± 0.20 (a)	11.73 ± 0.52 (c)	13.83 ± 0.07 (d)	17.00 ± 0.31 (e)	9.34 ± 0.02 (b)
4,5-Di-*O*-caffeoyl-quinic acid	6.71 ± 0.51 (ab)	6.25 ± 0.29 (a)	8.81 ± 0.05 (c)	14.72 ± 0.16 (d)	6.97 ± 0.02 (b)
Miscellanea					
Ascorbic acid	9.39 ± 0.10 (c)	0.58 ± 0.29 (b)	11.71 ± 0.04 (d)	15.52 ± 0.21 (e)	N.D.
Tryptophan	10.58 ± 0.03 (c)	10.33 ± 0.12 (b)	20.37 ± 0.06 (e)	11.25 ± 0.12 (d)	10.08 ± 0.10 (a)
Rutin	5.97 ± 0.12 (a)	9.05 ± 0.12 (b)	10.99 ± 1.21 (c)	14.15 ± 1.29 (d)	20.19 ± 0.37 (e)
Glycoalkaloids					
Solanidadienol triose	8.58 ± 0.06 (d)	5.7 ± 0.17 (a)	7.8 ± 0.71 (c)	6.82 ± 0.36 (b)	10.07 ± 0.28 (e)
Solanidenediol triose	0.86 ± 0.02 (ab)	0.93 ± 0.06 (b)	1.21 ± 0.08 (c)	1.38 ± 0.15 (d)	0.73 ± 0.07 (a)
Solanidadienol lycotetraose	2.47 ± 0.05 (a)	2.39 ± 0.20 (a)	2.92 ± 0.25 (b)	2.31 ± 0.13 (a)	4.74 ± 0.19 (b)
Solanidenol lycotetraose	2.21 ± 0.02 (a)	2.46 ± 0.15 (ab)	2.63 ± 0.21 (b)	2.64 ± 0.13 (a)	2.21 ± 0.08 (a)
Solanidenediol tetraose	0.93 ± 0.02 (b)	1.12 ± 0.09 (c)	1.23 ± 0.08 (c)	1.48 ± 0.08 (d)	0.34 ± 0.04 (a)
Dehydrodemissine	304.00 ± 5.75 (d)	95.3 ± 7.70 (a)	184.80 ± 14.04 (c)	193.00 ± 12.21 (c)	139.60 ± 4.19 (b)
Dehydrocommersonine	144.10 ± 4.20 (b)	95.2 ± 7.97 (a)	100.30 ± 9.70 (a)	102.00 ± 6.06 (a)	90.00 ± 2.88 (a)

[a]) The metabolite content represents the average of triplicate determinations (mean ± S.D.). [b]) Means with different letters in parentheses within the same row differ significantly by *Duncan*'s multiple range test ($P \leq 0.05$). [c]) N.D.: Not detected.

ions with peaks at m/z 884.52 ($[M-\text{Xyl}+\text{H}]^+$), 854.52 ($[M-\text{Glu}+\text{H}]^+$), 560.41 ($[M-\text{Xyl}-2\,\text{Glu}+\text{H})^+$), 398.31 ($[M-\text{Xyl}-2\,\text{Glu}-\text{Gal}+\text{H}]^+$), and 380.33 ($[M-\text{Xyl}-2\,\text{Glu}-\text{Gal}-\text{H}_2\text{O}+\text{H}]^+$), in agreement with the presence of lycotetraose as carbohydrate moiety and solanidine as aglycone [22][24].

Table 2. *LC/MS Analysis of* Solanum commersonii *leaf Extracts*

Compound	t_R [min]	Sugar[a])	$[M+H]^+$	MS^2 Ions
Solanidadienol triose	7.88	3-hex	898.53	736.45, 574.41, **412.33**, 394.32, 271.22, 253.20
Solanidenediol triose	8.28	3-hex	916.43	754.44, 592.43, **430.34**, 412.33, 271.22, 253.20
Solanidadienol lycotetraose	8.28	lyc	1030.56	**412.30**, 394.32, 271.22, 253.20, 182.05, 142.05
Solanidenol lycotetraose	8.40	lyc	1032.55	**414.33**, 271.23, 253.21
Dehydrocommersonine	8.52	com	1046.57	884.52, 560.40, **398.30**, 380.33 271.22, 253.20
Solanidenediol tetraose	8.68	4-hex	1032.53	1016.57, 754.41, 592.38, **430.34**
Dehydrodemissine	8.70	lyc	1016.56	854.52, 560.41, 884.50, 380.33, **398.30**, 271.23, 253.21

[a]) Sugar abbreviations: hex, hexose; lyc, lycotetraose; com, commertetraose.

Two less abundant solanidenediol derivatives were eluted at 8.28 and 8.68 min ($[M+H]^+$ ions with peaks at *m/z* 916.43 and 1032.53, resp.). Both compounds showed in their MS^2 spectra an ion with a peak at *m/z* 430.34, suggesting the presence of a solanidane-like aglycone carrying three O-atoms and having a C=C bond [23]. Information about the structure of the glycosides was inferred from the MS^2 spectra (*Table 2*). Fragmentation of the $[M+H]^+$ ion with a peak at *m/z* 916.43 showed daughter ions with peaks at *m/z* 754.44 ($[M-162+H]^+$), 592.43 ($[M-162-162+H]^+$), 430.34 ($[M-162-162-162+H]^+$), and 412.33 ($[M-162-162-162-H_2O+H]^+$). The presence of the fragment ions with peaks at *m/z* 271 and 253 indicated that two of the oxydril groups of the solanidane moiety appeared to be located on the *E*- and *F*-rings, which suggested that the GA was a solanidenediol triose ($C_{45}H_{73}O_{18}N$; calc. 916.49). The tandem mass spectrum of the ion with a peak at *m/z* 1032.53 ($C_{50}H_{81}NO_{21}$; calc. 1032.54) showed ions with peaks at *m/z* 754.41 ($[M-[132+146]+H]^+$), 592.38 ($[M-[132+146]-162+H]^+$), and 430.34 ($[M-[132+146]-162-162+H]^+$), suggesting that the carbohydrate chain (peak at *m/z* 602) contains a pentose, a deoxyhexose, and two hexoses conjugated to an aglycone similar to that described for the previous compound. We named this compound solanidenediol tetraose in analogy to the nomenclature reported by *Shakya* and *Navarre* [23]. Among the minor alkaloids, two solanidadienol derivatives were found ($[M+H]^+$ ions with peaks at *m/z* 898.53 and 1030.46, resp.) eluted at 7.88 and 8.28 min, respectively. Both compounds carry as aglycone a solanidine moiety with an O-atom and an additional unsaturation, as demonstrated by the daughter ion with a peak at *m/z* 412.3 observed in their MS^2 spectra [23]. Fragmentation of the compound showing a peak at *m/z* 898.53 generated ions with peaks at *m/z* 736.45 ($[M-162+H]^+$), 574.41 ($[M-162-162+H]^+$), 412.33 ($[M-162-162-162+H]^+$), and 394.32 ($[M-162-162-162-H_2O+H]^+$), suggesting the GA was a solanidadienol triose ($C_{45}H_{72}NO_{17}$; calc. 898.48). On the basis of similar data (*Table 2*), the latter appeared to be a solanidadienol lycotetraose. The alkaloid eluted at 8.4 min ($[M+H]^+$ ion peak at *m/z* 1032.55; ($C_{50}H_{79}NO_{21}$; calc., 1030.52) was identified as a solanidane derivative on the basis of the low relative abundance of the $[M-H_2O+H]^+$ ion in its MS^2 spectrum. Indeed, solanidane derivatives are not very prone to H_2O loss during fragmentation, while spirosolanes tend to be dehydrated yielding $[M+H-H_2O]^+$ as base ion [24]. This alkaloid eluting at 8.4 min showed an aglycone ion at *m/z* 414.33, corresponding to a solanidane with

two O-atoms, and a carbohydrate moiety analogous to that described for dehydrodemissine. Consequently, this compound was hypothesized as solanidenol lycotetraose [23].

Effect of Polyploidization. The quali-quantitative profiles of the leaf secondary metabolites of diploid *cmm 1T* and its synthetic tetraploids were determined to evaluate the effect of polyploidization. Both HPLC/UV and LC/MS profiling quickly revealed that there were no qualitative differences in the metabolite profiles among the analyzed genotypes. According to *Murray* and *Williams* [25] and *Levy* [26], induction of polyploidization may alter the secondary metabolites of a plant in a qualitative manner. To explain the changes in metabolite profiles occurring in polyploids, *Dhawan* and *Lavania* [14] proposed the presence of a disturbance of metabolic mechanisms that regulate the biosynthesis of individual compounds. It may be hypothesized that no functional repression or derepression of structural genes involved in the metabolic pathways of metabolites analyzed in our study actually occurred. By contrast, the diploid progenitor *cmm 1T* and the tetraploid genotypes displayed significant differences in the quantitative composition of secondary metabolites (phenylpropanoids and GAs) identified (*Table 3*). Comparing the quantity of phenylpropanoid compounds of

Table 3. *Mean Square and* F-*Values for the Analysis of Variance of the Secondary Metabolite Content in the Leaves of the* Solanum commersonii *Genotypes Used in this Study.* The data analyzed refer to both the diploid progenitor clone and its four tetraploid derivatives.

Source of variation	d.f.[a])	Mean Square	*F*-Value	
Phenylpropanoids				
Caffeoylquinic acid	4	10.93	68.42	**[b])
Caffeic acid	4	22.89	1018.92	**
Neochlorogenic acid	4	11.25	220.86	**
Chlorogenic acid	4	221.22	1143.88	**
Bis(dihydrocaffeoyl)spermine	4	3.86	85.64	**
Bis(dihydrocaffeoyl)spermidine	4	58.20	2102.61	**
Ferulic acid	4	43.48	760.83	**
Ferulic acid amide	4	80.19	105.50	**
Tris(dihydrocaffeoyl)spermine	4	39.04	474.19	**
4,5-Di-*O*-caffeoylquinic acid	4	36.86	495.90	**
Miscellanea				
Ascorbic acid	4	142.18	5156.31	**
Tryptophan	4	58.33	7628.62	**
Rutin	4	87.41	134.99	**
Glycoalkaloids				
Solanidadienol triose	4	8.33	56.27	**
Solanidenediol triose	4	0.22	28.15	**
Solanidadienol lycotetraose	4	3.13	98.35	**
Solanidenol lycotetraose	4	0.14	6.75	**
Solanidenediol tetraose	4	0.56	126.62	**
Dehydrodemissine	4	18232.12	199.89	**
Dehydrocommersonine	4	1398.44	31.75	**

[a]) d.f.: Degrees of freedom. [b]) **: Significant at $P \leq 0.01$.

our tetraploid genotypes to that of *cmm 1T* (*Table 1*), we found that the amount of caffeic and caffeoylquinic acids, tris(dihydrocaffeoyl)spermine, and bis(dihydrocaffeoyl)spermine was higher in tetraploids than in the diploid *cmm 1T*. The bis(dihydrocaffeoyl)spermidine and the 4,5-di-*O*-caffeoylquinic acid contents showed an increasing trend in the tetraploids, with the exception of genotype *cmm pt 15*. Among tetraploids, *cmm pt 24* showed contents of caffeic and caffeoylquinic acids, tris(dihydrocaffeoyl)spermine, and bis(dihydrocaffeoyl)spermidine *ca*. two to three times higher than the diploid progenitor (7.33, 15.17, 17.00, and 16.14 mg/100 g of dried tissue for *cmm pt 24 vs.* 2.88, 7.63, 7.90, and 8.88 mg/100 g of dried tissue for *cmm 1T*, resp.). Genotype *cmm pt 24* was also the only tetraploid with a 3-CQA content higher than *cmm 1T*. The ascorbic acid, tryptophan, and rutin contents of *cmm 1T* and its tetraploids were also investigated (*Table 1*). The 2*x* progenitor *cmm 1T* displayed a rutin content lower than all 4*x* derivatives. While the rutin content was *ca*. four times higher in *cmm pt 30* than in the diploid progenitor (20.19 mg/100 g of dried tissue for *cmm pt 30 vs.* 5.97 mg/100 g of dried tissue for *cmm 1T*), ferulic acid amide and ferulic acid displayed an increasing trend in the tetraploids, with the exception of *cmm pt 30*. Interestingly, the content of these metabolites was about twice as high in *cmm pt 24* as in *cmm 1T* (17.59 and 13.61 mg/100 g of dried tissue for *cmm pt 24 vs.* 9.52 and 6.71 mg/100 g of dried tissue for *cmm 1T*, resp.). Analysis of contrasts between *cmm 1T* and its tetraploids provided evidence that, except for 3-CQA, the average content of each phenylpropanoid compound was always higher in tetraploids (*Table 4*). Similarly, in *Camellia sinensis*, *Sardzhveladze* and *Kharebava* [27] observed an increased concentration of polyphenols, catechins, extractines, and caffeine in the leaf shoots of synthetic tetraploids with respect to the diploid progenitor.

In view of the role of phenylpropanoids in suppressing pathogens and in response to abiotic stresses [28–30], our results are interesting from the breeding standpoint. Indeed, tetraploid genotypes of *S. commersonii* are produced and used in breeding schemes aimed at overcoming sexual barriers [31]. Due to the complexity of the biosynthetic pathways of phenylpropanoids, including many branches and enzymes, we hypothesized that, after chromosome doubling, an increased number of genes may lead to an increased concentration and activity of some enzymes. Therefore, the flow through rate-limiting reactions increased and in turn may have provided additional substrate for other branches or steps of the biosynthetic pathway. In tetraploids that showed no increase in the phenylpropanoid content compared to diploid *cmm 1T*, gene silencing or other homeostatic devices (such as negative feedback mechanisms) may regulate and control the rate of metabolite synthesis to maintain a stable internal equilibrium. Moreover, alterations in metabolic biosynthesis can be due to complex gene regulatory networks controlled by several transcription factors that positively or negatively control the expression of one or a few phenylpropanoid biosynthetic steps.

Since *S. commersonii* falls within the group of potato species with the highest GA content [32], the effect of polyploidization on GAs undoubtedly merits investigation. As a family, the *Solanaceae* mainly present steroidal GAs derived from a cholestane skeleton and the steroidal alkaloids generally exist as glycosides. We found minor (solanidadienol triose, solanidenediol triose, solanidadienol lycotetraose, solanidenol lycotetraose, and solanidenediol tetraose) and major GAs (dehydrodemissine and dehydrocommersonine) with different aglycon moieties (*Table 1*). The contents of the

Table 4. *Analysis of Contrasts Between Diploid* (2*x*) Solanum commersonii *and Its Induced Tetraploid* (4*x*) *Genotypes in Terms of Leaf Secondary Metabolite Contents*

Metabolite	Content of 2*x vs.* 4*x*[a])	Mean Square	*F*-value	Significance[b])
Phenylpropanoids				
Caffeoylquinic acid	2.88 *vs.* 6.50	31.538	189.99	**
Caffeic acid	7.63 *vs.*11.38	33.735	1984.41	**
Neochlorogenic acid	4.49 *vs.* 4.52	0.002	0.04	n.s.
Chlorogenic acid	60.19 *vs.* 54.15	87.701	640.15	**
Bis(dihydrocaffeoyl)spermine	4.29 *vs.* 5.99	6.922	141.27	**
Bis(dihydrocaffeoyl)spermidine	8.88 *vs.* 10.94	10.226	511.30	**
Ferulic acid	9.52 *vs.* 12.42	20.080	542.70	**
Ferulic acid amide	6.71 *vs.* 8.17	5.098	7.03	*
Tris(dihydrocaffeoyl)spermine	7.90 *vs.* 12.99	62.098	926.84	**
4,5-Di-*O*-caffeoylquinic acid	6.70 *vs.* 9.19	14.821	302.47	**
Miscellanea				
Ascorbic acid	9.39 *vs.* 6.95	14.240	459.35	**
Tryptophan	10.58 *vs.* 13.01	14.084	2012.00	**
Rutin	5.97 *vs.* 13.58	138.746	182.56	**
Glycoalkaloids				
Solanidadienol triose	8.58 *vs.* 7.60	2.325	16.97	**
Solanidenediol triose	0.86 *vs.* 1.07	0.105	21.00	**
Solanidadienol lycotetraose	2.47 *vs.* 3.09	0.925	23.72	**
Solanidenol lycotetraose	2.21 *vs.* 2.49	0.177	11.80	**
Solanidenediol tetraose	0.93 *vs.* 1.04	0.031	10.33	*
Dehydrodemissine	303.90 *vs.* 153.18	54537.380	719.90	**
Dehydrocommersonine	144.10 *vs.* 96.94	5337.380	144.44	**

[a]) The metabolite contents were expressed as mg/100 g of dried tissue. [b]) Significance: **, significant at $P \leq 0.01$; *, significant at $P \leq 0.05$; n.s., not significant.

minor GAs ranged from 8.58 mg/100 g of dried tissue (solanidadienol triose in *cmm 1T*) to 0.34 mg/100 g of dried tissue (solanidenediol tetraose in *cmm pt 30*; *Table 1*). Among the minor GAs, the contents of solanidenediol triose, solanidadienol lycotetraose, solanidenol lycotetraose, and solanidenediol tetraose were higher in tetraploids than in the diploid progenitor *cmm 1T*, while that of solanidadienol triose showed the opposite trend (*Table 4*). The contents of the major GAs dehydrocommersonine and dehydrodemissine ranged between 303.9 mg/100 g (dehydrodemissine in *cmm 1T*) and 90.0 mg/100 g of dried tissue (dehydrocommersonine in cmm pt 30; *Table 1*). The contents of the major GAs were significantly higher in the diploid *cmm 1T* than in the tetraploids (*Table 4*). Similar results were reported in *Solanum chacoense* [33], where leaf concentrations of leptines and total GAs were significantly lower in 4*x* than in 2*x* genotypes. By contrast, an increase in the total alkaloid content following polyploidization has been reported in other species [34][35]. We found that major GAs had the same aglycone, *i.e.*, solanidine, whereas the minor GAs had a different aglycone moiety. Although the GA biosynthetic pathway and its genes and intermediates are not fully delineated [36], it can be hypothesized that GAs with a different aglycone moiety might be subject to different genetic control. Genetic and/or epigenetic changes due to

polyploidization may have caused modifications in the biosynthetic pathways of these compounds, increasing their oxidation pattern. Therefore, to balance their internal equilibrium after the modification of the ploidy level, our tetraploid genotypes of *S. commersonii* may have adjusted their enzymatic processes, slightly increasing the biosynthesis of the less abundant GAs to compensate for the disequilibrium among metabolites. From a breeding perspective, GA reduction is not desirable in the leaves, since the presence of GAs is often correlated with pest and disease resistance. By contrast, it is desirable in the tubers of commercial varieties, where the GA content should not exceed 20 mg/100 g of fresh weight [36].

Conclusions. – We believe that three main evidences emerged from this study. First, polyploidy induction significantly affected the phenylpropanoid and GA content of *S. commersonii* in a quantitative rather than a qualitative manner. Second, tetraploids generally showed an increased amount of phenylpropanoid compounds, suggesting that relevant genes may have a dosage dependency that may lead to a higher concentration and activity of some enzymes and/or transcription factors. Future research should explore these possibilities. Finally, tetraploids presented a lower leaf content of major GAs than the diploid progenitor. For these metabolites, there may well be a strong gene balance at the diploid level and gene duplication may have detrimental effects, as recently reviewed by *Birchler* and *Veitia* [37]. In the cultivated potato, the edible part is the tuber, whereas in this study, the GA content was investigated at the leaf level. Therefore, since GAs may display human toxicity and our tetraploid genotypes of *S. commersonii* are being used in breeding programs, it will be interesting to determine whether the GA level reduction detected in the leaves also occurs in the tubers of our synthetic *S. commersonii* tetraploids and in hybrid materials.

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Experimental Part

General. Anal.-grade hexane and MeOH were obtained from *Carlo Erba* (Milan, Italy). HPLC-Grade MeOH and HCOOH were purchased from *J. T. Baker* (*Mallinckrodt Baker*, Phillipsburg, NJ, USA). HPLC-Grade H_2O (18 mV) was prepared using a *Millipore* (Bedford, MA, USA) *Milli-Q* purification system. Oryzalin (=4-(dipropylamino)-3,5-dinitrobenzenesulfonamide) was obtained from *Duchefa* (NL-Haarlem). Standards for the identification and quantification of the compounds were purchased from *Extrasynthese* (F-Lyon) and *Sigma Aldrich* (I-Milan), and α-solanine, used as HPLC standard to perform the LC/MS/MS quant. analyses of the glycoalkaloids (GAs), was purchased from *Extrasynthese* (F-Lyon).

Plant Material. A clone (named *cmm 1T*) belonging to accession PI 243503 of diploid ($2n=2x=24$) *S. commersonii* was used in this study. It was kindly provided by Dr. *J. Bamberg* of the University of Wisconsin (Madison, USA), and was maintained and propagated *in vitro* on *MS* medium [38] adjusted to pH 5.8 and containing salts, vitamins, sucrose (30 g/l), and microagar (9 g/l). The cultures were maintained in a growth chamber at $25\pm2^\circ$ under a 14/10 (light/dark) photoperiod at 125 μJ m^{-2} s^{-1} irradiance provided by a cool, white-fluorescent tube (*Philips*). Two subcultures on the growing medium at 3-week intervals were necessary to obtain a sufficient number of fully developed plantlets for somatic

chromosome doubling. For the secondary metabolite analysis, three plants of each genotype were grown together under the same greenhouse conditions at 20–25° at natural light. The leaves were collected at the same date and the same physiological stage before flowering.

Polyploidy Induction and Verification. Tetraploids were induced by *in vitro* oryzalin treatment of non-flowering shoot tips (1 cm long) as described by *Chauvin et al.* [39]. The apical shoots of 40 plants of *cmm 1T* were dipped for 24 h in sterile and agitated H_2O (100 rpm at r.t.) containing 10 mg/l of the herbicide oryzalin (28.8 μM). The stock soln. of the herbicide was prepared by dissolving 40 mg/l of oryzalin in 1 ml of DMSO (*Sigma D5879*, USA). After the oryzalin treatment, the shoots were transferred to solidified *MS* medium as described above. After three weeks, the surviving oryzalin-treated plantlets were propagated and then transferred *in vivo* to a greenhouse for further analysis. A rough pre-selection of plants was performed. A few strips of epidermis were torn from the abaxial side of fully expanded leaves and then mounted on a microscope slide with a drop of dist. H_2O and a cover slip, to count the chloroplasts in the stomata cells. Plants with 10–12 chloroplasts were considered diploids, those with 20–24 chloroplasts tetraploids. The chromosome number of putative tetraploid plants was then assessed by mitotic chromosome analysis of root tip cells, according to *Carputo* [40].

Sample Preparation for Secondary Metabolite Analyses. A representative quantity (0.5 g) of fresh leaves was freeze-dried, defatted with hexane, and then extracted for 48 h with MeOH by exhaustive maceration (3×10 ml). Solns. (1 mg/ml) of each MeOH residue were prepared, and aliquots of 20 μl of each soln. were injected for analysis. Triplicate injections were made for each sample. To detect the secondary metabolite composition of the leaves, the sample preparation and HPLC-anal. conditions were adjusted to avoid any detectable degradation of these compounds during the performance. Stock solns. (10 mg/ml) of all standards were prepared in MeOH, except those of tyrosine and tryptophan that were prepared in 0.1N HCl.

To determine the GA content, a part of the MeOH extract was diluted to 10% MeOH at a concentration of 0.4 mg/ml. An appropriate volume of the internal standard (IS) α-solanine (in 2.5% metaphosphoric acid) was added to reach a final concentration of 0.1 μmol/ml, and a 100-μl aliquot was injected into the anal. system ($n = 3$). Samples were kept chilled and protected from bright light.

HPLC-DAD Analysis. The HPLC analyses were conducted using an *Agilent 1100* series system (*Agilent Technologies*, Palo Alto, CA, USA), equipped with a binary pump delivery system *G-1312*, a degasser *G-1322A*, a photodiode array detector *G-1315A* (DAD), an injection system *Rheodyne G-1328A*, and an *Onyx* monolithic C_{18} column (50×2.0 mm, particle size 2 μm, *Phenomenex*). The secondary metabolites were eluted with a gradient of *buffer A* (10 mM HCOOH with NH_4OH, pH 3.5) and *buffer B* (100% MeOH with 5 mM NH_4HCO_2) at a flow rate of 1 ml/min with the following program: 100% *A* for 1.0 min, gradient from 0 to 30% *B* in 5.7 min, then from 30 to 70% *B* in 5 min and from 70 to 100% *buffer B* in 2.86 min, and finally 100% *B* for 5.44 min. The injection volume was 20 μl, and the DAD was set at 210, 280, and 254 nm. An external standard method of calibration was used: for each standard, a six-point calibration curve was obtained. Neochlorogenic acid was evaluated as 3-CQA equivalent and the dihydrocaffeoyl polyamines as dihydrocaffeic acid equivalents at 210 nm without adjustment made for the polyamine part of the molecule [22][41].

LC/MS/MS Analysis. The LC/MS/MS analyses were performed with a *Q-TOF Premier* instrument (*Waters*, Milford, MA) equipped with an electrospray ion source (ESI (pos.)) and coupled to a *2690 Alliance* HPLC. The instrument accuracy was checked by external calibration with Glu-1-fibrinopeptide B (Glu-Fib). Instrument tuning and mass calibration of the spectrometer were performed using α-solanine ($[M+H]^+$ at m/z 869.07) as standard. MS/MS Data processing involved the use of MassLynx 4.1 software. Chromatographic separation was conducted by injecting 100 μl (40 μg) of each extract on an *Onyx* monolithic C_{18} column (50×2.0 mm, particle size 2 μm, *Phenomenex*) column. Gradient elution at a flow rate of 1 ml/min was carried out as described above (*cf. HPLC-DAD Analysis*). Mass analyses were performed using a dependent MS/MS function. The following instrumental parameters were used: source temp., 80°; desolvation temp., 180°; cone gas flow rate, 50 l/h; desolvation, 300 l/h; capillary voltage, 3 kV; cone voltage, 28 V; extraction cone, 5; fragmentation voltage, 40 V. The MCP detector was set at 2250 V, and the energy adjustable collision cell was filled with pure Ar. The full mass scan range was between m/z 250 and 1500.

Statistical Analysis. Statistical analyses were performed using the 2002 Statistical Package for Social Sciences (SPSS) version 11.5 for *Windows*. Analysis of variance was used to evaluate the differences in the metabolite content among all genotypes. Mean separation was performed using *Duncan*'s test and referring to $P \leq 0.01$ and $P \leq 0.05$ probability levels.

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