

Polianthes Tuberosa as Edible Flower: Vitro Propagation and Nutritional Properties

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Abstract Tuberose (*Polianthes tuberosa*) is a bulbous species belonging to Asparagaceae family, characterized by a very high ornamental value, pleasant flower scent and taste. For all these reasons, flowers are currently used to produce perfumes or valorized as actual ingredient of different recipes, since they are completely edible. *P. tuberosa* is one out of 40 species studied in INTERREG ALC275 to extend the use of edible flowers as functional food and enlarge the number of the species used for the supply chain of the edible flowers. Fresh flowers were analyzed in order to characterize them from the nutrition point of view. They show good polyphenols content and antioxidant activity (DPPH assay), while flavonoids, anthocyanins and carotenoids amounts are low. On the other hand, *P. tuberosa* flowers can be a real source of vitamin C, because of the high quantities of this molecule in the petals. Soluble sugars are present in small amount. High quantities of *P. tuberosa* flowers are difficult to obtain due to low bulb multiplication rate and poor seed germination. For these reasons, in vitro culture was performed to facilitate plant propagation. Bulbs were surface-sterilized and germinated while seeds were surface-sterilized and germinated in agar solution. Both were rinsed twice with autoclaved distilled water for 10 minutes after sterilization. The bulbs were cultured in jars containing MS agarized substrate enriched with 3% sucrose, 1.5 mg/L BA, 0.5 mg/L IAA and 0.7% agar (pH 5.8). Seeds were germinated in Petri dishes with agar-water substrate and then 14 different clones were selected and cultured in jars. The multiplication rate of the clones was very variable but some of them reached a good multiplication rate.

The leaves are elongated, arched and ribbon-shaped. At late summer, the plants produce spikes, where the flowers develop starting from the bottom to the top. The white pleasant flowers are characterized by a long vase-life (around 10 days) [2], with very high ornamental value, an intense and sweet fragrance, floral taste and crunchy texture. Floral aroma scent volatile changes during flowers lifespan, showing a nocturnal rhythm [3]-[5]. For all these reasons, flowers are currently used for producing perfumes [6] or valorized as actual ingredient in different recipes, since they are completely edible [7]. In Asia, bulbs and flowers are used to extract compounds with anti-inflammatory and antispasmodic, diuretic and emetic properties [8].

P. tuberosa is one out of 40 species studied in INTERREG ALC275 to extend the use of edible flowers as functional food and enlarge the number of the species used for the supply chain of the edible flowers. Organically grown edible flowers are currently sold in rigid plastic containers, that can be purchased directly from the producers, both locally and through dedicated websites. In both cases, significant quantities of flowers are required to cover the demand. In fact, edible flowers are gaining more and more popularity thanks to many scientific reports, that highlight their nutritive and healthy properties [9][10]. Since few information are available about primary and secondary metabolites present in *tuberosa* flowers and [11], the main objective of this work was to analyze their total polyphenols, carotenoid, and ascorbic acid content, along with the quantification of their radical scavenging activity (DPPH assay) and soluble sugars (D-glucose, D-fructose and sucrose) concentration, in order to better characterize the flowers from the nutritional point of view.

Index Terms Edible flowers, micropropagation, antioxidant, bioactive compounds, DPPH assay, vitamin C

I. INTRODUCTION

Polianthes tuberosa is an ornamental bulbous plant belonging to Asparagaceae family and native to Mexico [1]. The bulbs produce flowers only once in their life, and each bulb grow an average of four years before producing. On the other hand, high quantities of tuberose flowers are difficult to obtain due to low bulb multiplication rate and poor seed germination. For these reasons, in vitro cultivation techniques are performed in this work to extend the use of edible flowers as functional food and enlarge the number of the species used for the supply chain of the edible flowers. Organically grown edible flowers are currently sold in rigid plastic containers, that can be purchased directly from the producers, both locally and through dedicated websites. In both cases, significant quantities of flowers are required to cover the demand. In fact, edible flowers are gaining more and more popularity thanks to many scientific reports, that highlight their nutritive and healthy properties [9][10]. Since few information are available about primary and secondary metabolites present in *tuberosa* flowers and [11], the main objective of this work was to analyze their total polyphenols, carotenoid, and ascorbic acid content, along with the quantification of their radical scavenging activity (DPPH assay) and soluble sugars (D-glucose, D-fructose and sucrose) concentration, in order to better characterize the flowers from the nutritional point of view.

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II. MATERIALS AND METHODS

A. Plant Material

Seeds and bulbs of *P. tuberosa* were provided by two growers of perfume plants: Sebastien Rodriguez (bulbs in pot and seeds) and Armelle Janody (only seeds). Thirty bulbs deriving from a single clone were cultured in 9-liter pots (30 cm of diameter) containing substrate (Hochmoor ± Terflor, Capriolo, BS, Italy) with slow release fertilizer (Nitrophoska, Eurochem Agro, Cesana Maderno, MB, Italy) were grown organically without the use of pesticides and irrigated with nutrient solution (Ferti 3, Planta- Q J H P L W W H O 5 H J H Q V W a u r, Germany) every week. The pots were kept in a greenhouse with anti-insect net and located at CREA Research Centre for Vegetable and Ornamental Crops (CREA, Sanremo, IM, Italy, GPS: 43.816887, 7.758900).

Forty bulbs of this clone were used to establish the *in vitro* culture, the remaining bulbs were used to produce edible flowers. Thirty seeds were stored at room temperature until *in vitro* seeding.

B. Flower Analysis

Full-bloomed flowers were harvested in summer 2018 and immediately stored at -80 °C until analyses.

Flower colour was evaluated with a spectrophotometer SP60 series (X-Rite Incorporated, Michigan, USA), measuring L* (lightness), a* (redness) and b* (yellowness) colour coordinates (CIELAB scale, CIE 1976). Twelve different and well representative measures were recorded in order to determine the flowers colour.

Frozen flowers (200 mg) were used to quantify total carotenoid content, total polyphenolic content, Total Flavonoid (TF) and anthocyanin content, as previous described [12]. Radical scavenging activity was determined by the DPPH assay [13], reporting the results as IC₅₀ (mg/mL). Total and the reduced form of ascorbic acid were quantified according to the method of Reference [14]; sample extraction was performed following the protocol already described in literature [15]. Sucrose, Fructose and D-glucose determination was performed using a Sucrose/D-glucose Assay Kit (Megazyme International Ireland, Co. Wicklow, Ireland, UHODQG IROORZLQJ WKH PDQGHU, Ireland) prior to the extraction described in [16]. (Fig. 1).

For each analysis, three biological replica were used. The absorbance was read in a UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan).



Figure 1. Inflorescence of *P. tuberosa* clone grown in pot.

C. Bulbs and Seeds Surface Sterilization

Forty bulbs were extracted from the pot, washed under running tap water to remove soil residues, surface-sterilized in a 70 % (v/v) ethanol solution (R U a n f f) then transferred in a 2.5 % (v/v) sodium hypochlorite (NaClO) solution (R U c l o n e B). In parallel, thirty seeds were surface-sterilized 1 % (v/v) NaClO solution (R U c l o n e C). Bulbs and seeds were rinsed twice with autoclaved distilled water for 10 minutes after sterilization.

D. In Vitro Culture of Bulbs and Seeds

The bulbs were cultured *in vitro* in a jar containing 12 ml of MS propagation medium [7] agarized substrate enriched with 3% sucrose, 1.5 mg/L BA, 0.5 mg/L IAA and 0.7% agar (pH 5.8) [18] and kept at 23 ± 1 °C with a 16/8 h light/dark cycle and 209 ± 5 μmol photons m⁻² s⁻¹ irradiance at tube level (white fluorescent Philips Master TL-D 36W/840 lamp). Only one bulb (B clone) was selected for subsequent propagation subculture in jars (Fig. 2).



Figure 2. *In vitro* culture of B clone in jar (bar = 1 cm).

The seeds were kept in Petri dishes containing agar-water substrate (pH 5.8) and placed to germinate in the dark at 23 ± 1 °C. Seed germination was checked weekly. After three months, the plants originated from seeds (S clones) were transplanted in jars containing propagation medium [18], already used during B clone multiplication phase. After one year of subculture, the multiplication rate was recorded, namely the number of propagules deriving from a single plant of a clone (Fig. 3).

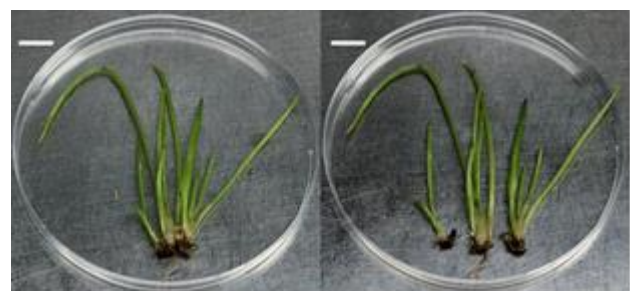


Figure 3. Evaluation of multiplication rate for S4 clone (bar = 1 cm).

E. Acclimatization in Greenhouse

In spring, a total of 500 bulbs, obtained from four different in vitro clones with the highest multiplication rate, were acclimatized in cellular containers containing peat: perlite 1:1. The plants were watered if necessary and cultured in greenhouses at CREA of Sanremo and other S and the B clone. The S7 clone had the lowest CREAM of Nice Cedex

After three months, plant survival was recorded.

F. Statistical Analysis

Data were statistically analyzed by one-way ANOVA. The significance of the comparisons was estimated by a Tukey difference test with cutoff significance at $p < 0.05$.

III. RESULTS

A. Flower Analysis

P. tuberosa flowers analysed in this work were very closed to white ($a^* e b^*$ coordinates not far from 0), with a high brightness value (L^*).

TABLE I. CIE L*A*B* COLOUR PARAMETERS DATA ARE REPORTED AS MEANS ± STANDARD ERROR (SE) (N = 12).

Color specification	Means ± SE
L*	91.88 ± 0.19
a*	-2.10 ± 0.03
b*	8.27 ± 0.18

Secondary metabolites are differently represented depending on the class of compounds. Good amount of total polyphenols was quantified in *P. tuberosa* flowers, while the total flavonoid content was 5.3-fold lower. Anthocyanins were very limited (0.02 mg/g FW) as well as total chlorophylls and carotenoids.

On the other hand, flowers antioxidant activity was relevant. Significant concentration of ascorbic acid (vitamin C) was determined, and the reduced form of this molecule was very high (94.5%) compared to the oxidized one (5.5%, calculated by subtracting the percentage of the reduced ascorbic acid from the percentage of the total ascorbic acid content, set at 100%).

As regards primary metabolites, low amounts of soluble sugars were quantified, and the two monosaccharides measured in this work were around 1.6-fold higher than sucrose.

TABLE II. DETERMINATION OF SECONDARY METABOLITES, ASCORBIC ACID, RADICAL SCAVENGER ACTIVITY AND SOLUBLE SUGARS DATA ARE REPORTED AS MEANS ± STANDARD ERROR (SE) (N = 3). ABBREVIATION: FW ± FRESH WEIGHT; GAE-GALLIC ACID EQUIVALENTS; CE ± CATECHIN EQUIVALENTS; ME- MALVIN EQUIVALENTS

Parameters	Means ± SE
Total chlorophylls (µg/ g FW)	3.97 ± 0.86
Carotenoids (µg/ g FW)	2.33 ± 0.39
Polyphenols (mg GAE/g FW)	3.53 ± 0.10
Flavonoids (mg CE/ g FW)	0.66 ± 0.03
Anthocyanins (mg ME/g FW)	0.02 ± 0.00
DPPH (IC ₅₀ mg FW/ml)	1.61 ± 0.05
Reduced ascorbic acid (mg/100g FW)	73.91 ± 0.91
Total ascorbic acid (mg/100g FW)	78.23 ± 0.95
Glucose (mg/g FW)	4.74 ± 0.48
Fructose (mg/g FW)	4.47 ± 0.46
Sucrose (mg/g FW)	2.80 ± 0.29

B. Multiplication in in Vitro Culture

The multiplication rate of the clones was very variable. The average multiplication rate of each clone is shown in Fig.4. S4 and S13 clones show the statistically significant highest average multiplication rate compared to other S and the B clone. The S7 clone had the lowest multiplication rate.

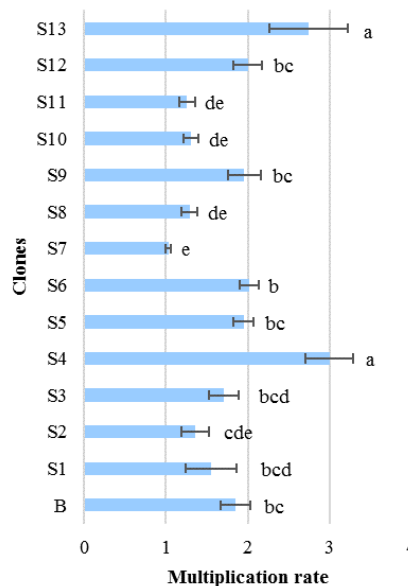


Figure 4. Average multiplication rate of *P. tuberosa* clones. B, clone from bulb; S, clones from seeds. Different letters indicates statistically significant differences at $p < 0.05$ (Tukey difference test).

C. Acclimatization in Greenhouse

No difference in acclimatization rates was highlighted between the two greenhouse sites (CREA and CREAM) (data not shown). The acclimatization of *P. tuberosa* bulbs from their vitro culture has not been difficult. Indeed, almost all bulbs of the four clones (B, S4, S6 and S13) survived the acclimatization phase. Percentages were 99.3 %, 99.4 %, 100.0 % and 98.9 % for B, S4, S6 and S13, respectively.

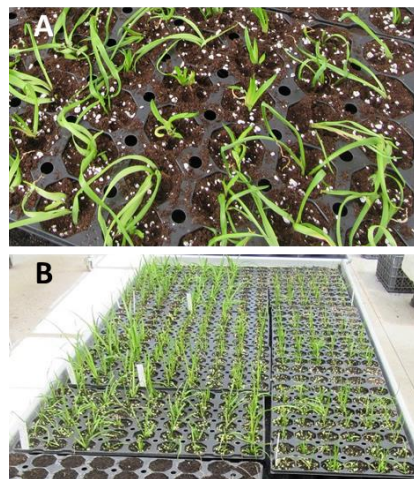


Figure 5. Acclimatization of the *P. tuberosa*. A. Bulbs from their vitro culture just transplanted in greenhouse at CREAM of Nice Cedex. B. Bulbs in cellular containers after three months in vivo culture.

IV. DISCUSSION

Even though *P. tuberosa* flowers are edible and traditionally consumed in different culinary preparations [7], poor information about their nutritive and antioxidant properties are reported in literature. When flowers of *P. tuberosa* L. (ecotype India) were extracted in methanol, reference [1] measured 180 mg of total polyphenols per gram of fresh weight (FW) along with a very high antioxidant activity (DPPH IC₅₀ value = 0.006 mg FW/ml). This value is quite far from the ones obtained in this work: 3.53 mg GAE/g FW and 1.61 mg FW/ml (DPPH IC₅₀), respectively. This discrepancy could be due to differences in: 1) extraction methods; 2) plant material genotypes; 3) plant growth condition. In fact, reference [1] performed flower extraction in a X O W U D V R Q L F E D W K R I Q with pure methanol as solvent. In this work, no sonication were carried out and the extraction solvent was a 70% (v/v) methanol solution. Moreover, reference [1] grown their plant in a experimental garden, instead of a greenhouse as in our work. This means that environmental conditions could be different, even if the season in which the experiment was performed and the age of the plant were comparable in the two works.

Carotenoids and anthocyanins are the major pigments of petals. Hybrids of tuberose with yellow flowers owe their colour to carotenoids, while anthocyanins confer pink, reddish-purple and purple flowers coloration [9]. The coexistence of anthocyanins and carotenoids allows to obtain orange flowers [9]. On the other hand, neither carotenoids nor anthocyanins are present in tuberose varieties with white flowers [9]. Despite this previous work, little quantities of both pigments are measured in our study (Tab.1), that involved white flowers. Based on flowers observation to the naked eyes, yellow anthers and filaments could contain carotenoids, while anthocyanins could be responsible for the slight pink shades sometimes present on the petals.

Ascorbic acid is an important nutrient for the human diet, and some edible flowers contains relevant quantities of this vitamin such as *Tagetes tenuifolia* (241.20 mg/100 g FW) [20]. Even if *P. tuberosa* flowers contain around 3-fold lower amount of ascorbic acid than the abovementioned species, its flowers can be a real source of this molecule since 25 g of petals (around 10 flowers) are enough to reach a quarter of the recommended daily intake for vitamin C [21].

Moreover, *P. tuberosa* flowers contain more ascorbic acid than other well-known edible species, such as *Borago officinalis*, *Bellis perennis* and *Begonia semperflorens* [20].

Regarding to soluble sugars, tuberose flowers are characterized by low amount of D-glucose, D-fructose and sucrose, compared to 4 different edible flowers belonging to *Agastache* family [22]. Nevertheless, *P. tuberosa* contain a higher sucrose percentage than 3 out of the 6 species analysed by reference [22].

As regards in vitro propagation techniques the multiplication rates were different among clones. Obviously, genetic variability is a fundamental aspect, since some genes present in a clone could change the

response to the hormones present in the culture medium. This trend is observed in other species. A study carried out on *Nerine*, another bulbous ornamental plant, shows that different clones placed on the same in vitro medium have very different multiplication rates [23]. Also in tree species, like oak and myrtle, the researchers noted large variations of shoot productivity among clones and varieties, although under the same multiplication conditions [24], [25]. The selection of plants that display a good in vitro multiplication aptitude improves the commercial potentiality.

Epigenetic factors inherited from parents can affect the rate of in vitro multiplications. Some authors [26] state that, in plant tissue culture, DNA methylation and histone modifications can affect shoot proliferation and quality propagules.

Acclimatization is in two steps: bulbs transplant and then bulb sprouting. With this system acclimatization percentages are excellent.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Formal analysis, Funding acquisition: B.R., L.P.; Investigation, Methodology, Project administration: B.R.; Supervision: B.R., L.P.; Writing original draft: A.C., I.M.; all authors had approved the final version.

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Aramon and in 2015, in the national experimental program 'Process Approach for the Commercial Innovation in Cut Flowers, Leaves and Twigs' at the Alpes Maritimes Chamber of Agriculture. In 2017 she won an award for being in the top ten in a national competition 'Young Ideas for Innovation in Agriculture in Southern Italy' with the theme of edible flowers. The competition was organized by Rete Rurale Nazionale 2014/2020 and MIPAAF. Rosanna is currently working at the Alpes Maritimes Chamber of Agriculture in Nice, France, on the Interreg Alcotra ANTEA program: 'Edible Flowers: Innovations for the Development of a Cross-Border Sector'. Her current research interests are in the field of edible flowers, agronomy and aromatic and medicinal plants.