Variability for characters related to inulin accumulation in topinambur (*Helianthus tuberosus L.*).

Mario Baldini, *Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, via delle Scienze, 208, 33100 Udine, Italia*,
Fax: +39 0432 558603 E-mail: baldini@palantir.dpvta.uniud.it

Francesco Danuso *Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, via delle Scienze, 208, 33100 Udine, Italia*,
Fax: +39 0432 558603 E-mail: danuso@palantir.dpvta.uniud.it

Maurizio Turi, *Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, via delle Scienze, 208, 33100 Udine, Italia*,
Fax: +39 0432 558603 E-mail: turi@palantir.dpvta.uniud.it

GianPaolo Vannozzi *Dipartimento di Produzione Vegetale e Tecnologie Agrarie, Università di Udine, via delle Scienze, 208, 33100 Udine, Italia*,
Fax: +39 0432 558603 E-mail: vannozzi@dpvta.uniud.it

Abstract

Today, Jerusalem artichoke (*Helianthus tuberosus* L.) is considered a sugar crop, especially for their fructose and inulin (fructose polymer) yield, used in many food and no-food applications. Instead of an annual crop for tuber yield, as used to date, a Jerusalem artichoke ideotype for stalk production, as a multi-year crop with an easier harvest mechanisation, could be of interest. With the above aim, seventy-eight new clones of Jerusalem artichokes (*Helianthus tuberosus* L.) were obtained at the University of Udine and investigated under north-eastern Italian conditions to determine the existing variability for several characteristics. In addition, four of these clones were compared, on hill plots, with a commercial cultivar (Violet de Rennes) as a control, in terms of sugar accumulation in stems and tubers, and stem and tuber yield at two harvest times. The population showed a large variability in flowering time and, in particular, a difference of 38 days between the earliest and the latest clone was found. Leaf photosynthesis and total soluble sugars in the stems (measured by refractometer) ranged from 9.4 to 31.2 µM CO_2 m^{-2} sec^{-1} and from 9.5 to 32.4%, respectively. Of the clones, the 70 yielded both the highest stem dry matter production and sugar content in the stems, opening interesting production perspectives, while Violet de Rennes confirmed its high tuber yield potential. Further studies will be carried out to confirm these preliminary results and to obtain relationships between refractometer index and inulin content, in order to utilise the refractometer as a rapid and early screening method to select genotypes with high inulin content.

Key words: Helianthus Tuberosus L., Jerusalem artichoke, Topinambur, breeding, inulin production.

Introduction

Today, Jerusalem Artichoke (*Helianthus tuberosus* L.) cannot be considered only as a wild species to be used in improving disease resistance in cultivated sunflower (Sackston, 1992; Cassels and Walsh, 1995), but as a cultivated crop to produce sugars, (especially fructose) and fructans (inulin), which are used as food or for various chemical, electronic and pharmaceutical applications

(Marchetti, 1993). Inulin is the polymer of fructose contained in Jerusalem artichoke (*Helianthus tuberosus* L.); in some Italian environments, the total sugar yield was about 6-7 t/ha, 70-80% of which in the form of fructose (Paolini *et al.*, 1996). The agronomic and economic interest in such a novel crop for sweeteners, would open new opportunities in improving and/or creating new varieties by breeding. In fact, at present, as an inulin production crop, just clones and "commercial varieties", improved for tuber production, are used. However, Jerusalem artichokes also store an important, if temporary, amount of fructans in the stalk (Incoll and Neales, 1970; Meijer and Mathijssen, 1991;), and so it could be proposed as an ideotype for a stalk crop, selected for the high sugar presence in the stalk, as a multi-year crop (Caserta and Cervigni, 1991b). The advantages of the crop for stalks are the simplification of the harvest (easier mechanisation) and the possibility of making it a multi-year crop.
The main objectives for developing new varieties are the maximisation of economic yield (sugars) in the stem, which has to substitute the tuber as a "sink" for inulin, and the production of large amounts of biomass. In order to obtain this new ideotype, at least two suitable selection indices are required for the breeding programme: one related to biomass production, which could be the elevated carbon exchange rate (Kosaric *et al.*, 1984; Lambers,1987; Soja and Haunold, 1991) and the other related to the inulin content in the plant organs. For the latter, a quick and easy method, avoiding time consuming and expensive laboratory analysis, is required. For the above reasons, the aim of this work was to create a new clone collection of *Helianthus tuberosus* L., obtained by hand crosses between wild and cultivated varieties and by seed reproduction, and to study their genetic variability for flowering time, stem and tuber soluble sugar accumulation, carbon exchange rate and biomass yield.

Material and Methods

In 1996-97, in order to create a new collection of *Helianthus tuberosus* clones, several crosses were made between "commercially available varieties" (Violet de Rennes, C146) and some local clones, or wild types (coded as FVG-CA, FVG-FO and FVG-UD). Some other new accessions were obtained by seed reproduction of the same genotypes. In 1998, the collection amounted to 79 clones.
The experiment was carried out in 1998 at the Experimental Farm of the University of Udine, in a loamy-sandy and shallow soil. Fertilisation, applied before sowing, consisted of 150 kg ha^{-1} of N, 100 kg ha^{-1} of P_2O_5 and 100 kg ha^{-1} of K_2O. 200 g of seed tubers per clone were planted by hand on March 25, in hill plots (20 cm deep) with the spacing between plants 2 m between rows and 2 m on rows, with a sowing density of 0.25 plants m^2. The experimental scheme was a completely randomised block design with two replications (hill plot). Half of each plot was used to determine production of the above-ground biomass and tubers at the first harvest time, while the other half was used for the determination of tuber production. Irrigation was applied to maintain the soil at non-limiting water conditions during the entire crop cycle.
On all the genotypes, the following parameters were determined:

- flowering time (as days of the year) corresponding to the R5.5 stage (Schneither and Miller, 1981);
- leaf photosynthesis (μM CO_2 m^{-2} sec^{-1}) during flowering time on fully developed upper leaves, utilising an open portable gas exchange instrument (LICOR 6400 IRGA). Measurements were carried out from noon to 3 p.m. (09/29/1999), without replications, at 1700 PPFD (μE m^{-2} sec^{-1});
- "refractometrically measured" solids, performed by a digital refractometer " mod. Palette 101" (ATAGO), on extracted (portable hydraulic press) tissue juice, during flowering time (09/30/1999). The amount of soluble components in the Jerusalem artichoke juice was expressed as the Brix-value: this is the total sugar concentration in % (w/v) of a solution.

Differences in the susceptibility to oidium infection was recorded for each clone.

For the clones, coded as 17, 22, 56, 70 and Violet de Rennes as a control, the time course of the total soluble sugar concentration were measured on 15th and 30th September, 30th October and 30th November. Moreover, for the same clones, the above-ground biomass (stem) and the tuber yield at two harvest times (30th September–harvest time for a stem crop and 30th November–final harvest for a normal tuber crops), were recorded.

Results and Discussion

The distribution of flowering time, photosynthesis and Brix values is presented in Figures 1a, b and c. The average flowering time for the population was 268 ± 6.9 (standard deviation), expressed as days of the year, corresponding to September 25th with a distribution skewed towards higher values for flowering time. The difference between the earliest (08/28) and latest (10/05) clones was 38 days; this large variability could allow the selection of genotypes for very different environments. Figure 1b shows the photosynthesis activity of the upper leaves at flowering time. The population showed an average value of 22.2 ± 4.86 μM CO_2 m^{-2} sec^{-1}, with two genotypes exceeding 30 μM CO_2 m^{-2} sec^{-1}, as was the case for clone 70 (31.2 μM CO_2 m^{-2} sec^{-1}), which could open interesting prospects. Many authors have shown that the high photosynthetic rate is unrelated to final tuber yield (Soja and Haunold, 1991) because of the importance of tuber sink capacity effects and other traits on the final economic yield. However, high rates of photosynthesis have been shown to determine outstanding biomass productivity in *Helianthus tuberosus* (Kosaric *et al.*, 1984) and this could be very relevant when the main storage capacity organs for inulin, in a new ideotype, could become the stem rather than the tuber.
The population variability for the refractometer values (Figure 1c) showed mean value of 22.9 ± 6.4, and a large range of variability in the population (from 9.5 to 32.4 as Brix values %). This variability is of fundamental importance for selecting genotypes with high fructan content in the stem, especially if a relationship between total soluble sugars, detected with the refractometer, and the inulin content in the plant organs in Jerusalem artichokes was found, as has already been described in chicory roots (Van Waes *et al.*, 1998).
Considering the time course of the refractometric index in the stem, clone 70 had the highest sugar content (Figure 2a) in correspondence with the time of flowering, when, normally, a stem crop is harvested. At the tuber harvest time, these differences disappeared and the main part of the sugars are transferred to the tubers.
The genotypes showed no significant differences in sugar content in the tubers, with the exception of clone 17 (Fig.2b), which had the lowest sugar concentration. Genotypes 22 and 70 delayed the tuberization with respect to the other genotypes; in fact, in correspondence to the first sampling date, only stolons were present (Fig.2b). This delay was probably due to the susceptibility of Jerusalem artichokes to photoperiod, as reported by Soya and Dersch, (1993).
The dry stem weight trend was similar in all genotypes, with a decrease from the flowering to tuber harvest time. Of the clones, clone 70 had a significantly higher stem biomass than the other genotypes at both harvest times (Fig.3a).
The Violet de Rennes had the highest tuber yield (Fig.3b) with respect to the other genotypes at both harvest times, confirming its aptitude to elevated economic yield production.

Conclusions

This preliminary study demonstrated a fairly wide variation in some plant characters strictly related to the inulin accumulation in a newly constituted collection of Jerusalem artichokes obtained at the University of Udine. In fact, knowledge of the existing range of variability in a available germoplasm for different characteristics is essential in any successful breeding programme.
In the new clone population (where Violet de Rennes was present as a control), number 70 had the highest photosynthesis activity, stem biomass and sugar concentration in the stem at flowering time.

This is an important result for the development of new cultivars of Jerusalem artichokes suitable as a crop for stalks and not for tubers, with a simplification of the harvest (easier mechanisation) and the possibility of making a multi-year crop.
The total soluble sugar concentration, measured by refractometer, determines only the soluble free sugars, which represent no more than 10% of the total sugar present in Jerusalem artichoke juice (Zubr and Pedersen, 1993; Kiehn and Chubey, 1993; Paolini *et al.*, 1996). The next objective is to find a relationship between the refractometer values and the total sugar content (polysaccharides as inulin), measured by standard procedures (analysis by HPLC are in progress). The presence of this relationship, already found in chicory roots by Van Waes *et al.*, (1998), but not applicable to Jerusalem artichoke tubers (data not shown), could permit (in a dynamic model of carbohydrate) the utilisation of refractometer values as a screening index to select genotypes for high inulin content.
At the same time, in the near future, studies on dynamic sugar accumulation in different organs of the plants during the day, the effect of climatic conditions and cultural practice management on the degree of polymerisation of the inulin chain will be carried out. Efforts will be also made to develop models to simulate Jerusalem artichoke crops under practical conditions, adapting and calibrating an existing simulation model for cropping systems (Danuso, 1997).

Acknowledgements
This research is supported by Murst national project "Crops for inulin production: modelling environmental effects and cropping strategies", co-ordinator Fancesco Danuso.
The authors thank Dr. Sattar Tahmasebi Enferadi for his technical assistance.

References

CASERTA G. and CERVIGNI T., 1991b. The use of Jerusalem artichoke stocks for the production of fructose and ethanol. Bioresource Technol., 35, 247-250.

CASSELLS A.C., WALSH M., 1995. Screening for Sclerotinia resistance in Helianthus tuberosus L. (Jerusalem artichoke) varieties, lines and somaclones, in the field and in vitro. Plant pathology, 44:428-437.

DANUSO F., FRANZ D., BIGOT L., BUDOI G., 1999. CSS: a modular software for cropping system simulation. Proc. Of Int. Symposium "Modelling cropping system", ESA, LLEIDA, 21-23 June, 1999- Spain.

KIEHN F.A., CHUBEY B.B., 1993. Variability in agronomic and compositional characteristics of Jerusalem artichoke. In A. Fuchs (Ed.), Inulin and Inulin-containing Crops, Elsevier Science Publishers B.V., 1-9.

KOSARIC N., COSENTINO A., WIECZOREK A., DUVNJAK Z. 1984. The jerusalem artichoke as an agricultural crop. Biomass, 5, 1-36.

INCOLL L.D., NEALES T.F., 1970. The stem as a temporary sink before tuberization in *Helianthus tuberosus* L. J. Exp. Bot., 21, 469-476.

PAOLINI R., DEL PUGLIA S., ABBATE V., COPANI V., DANUSO F., DE MASTRO G., LOSAVIO N., MARZI V., MOLFETTA P., PIGNATELLI V., VENEZIA G., VONELLA A.V., 1996. Produttività del topinambur (Helianthus tuberosus L.) in relazione a fattori agronomici diversi. Agricoltura Ricerca, anno XVIII, 163, 126-144.

LAMBERS H., 1987. Does variation in photosynthetic rate explain variation in growth rate and yield? Neth. J. Agric. Sci., 35, 505-519.

MARCHETTI G., 1993. Inulina e fruttani. Industrie Alimentari, XXXII, 945-949.

MEIJER, W.J.M., E.W.J.M. MATHIJSSEN, 1991. The relation between flower initiation and sink strength of stems and tubers of Jerusalem artichoke. Neth.J.Agric.Sci. 39: 123-135

SACKSTON W.E. 1992. Managing the major sunflower diseases: from cultural practices to breeding for resistance. In Proc. the 13th Int. Sun. Conf., Pisa, Italy, 7-11 September 1992, Pacini Editor, vol. II, 1533-1544.

SCHNEITER A.A., MILLER J.F. 1981. Description of sunflower growth stages. Crop Sci., 21, 901-903.

SOJA G., HAUNOLD E., 1991. Leaf gas exchange and tuber yield in Jerusalem artichoke (*Helianthus tuberosus*) cultivars. Field Crop Res., 26, 241-252.

SOJA G., DERSCH G., 1993. Plant development and hormonal status in the Jerusalem artichoke (Helianthus tuberosus L.). Industrial crops and products, 1, 219-228.

VAN WAES C., BAERT J., CARLIER L., VAN BOCKSTAELE E., 1998. A rapid determination of the total sugar content and the average inulin chain length in roots of chicory (*Cichorium intybus* L.). J. Sci. Food Agric., 76, 107-110.

VOLTOLINA G., 1994. Valutazione di specie diverse per la produzione di fruttosio e inulina. L'informatore Agrario, 33, 25-32.
ZUBR J. and PEDERSEN H.S., 1993. Characteristics of growth and development of different Jerusalem artichoke cultivars. In A. Fuchs (Ed.), Inulin and Inulin-containing Crops, Elsevier Science Publishers B.V., 11-19.

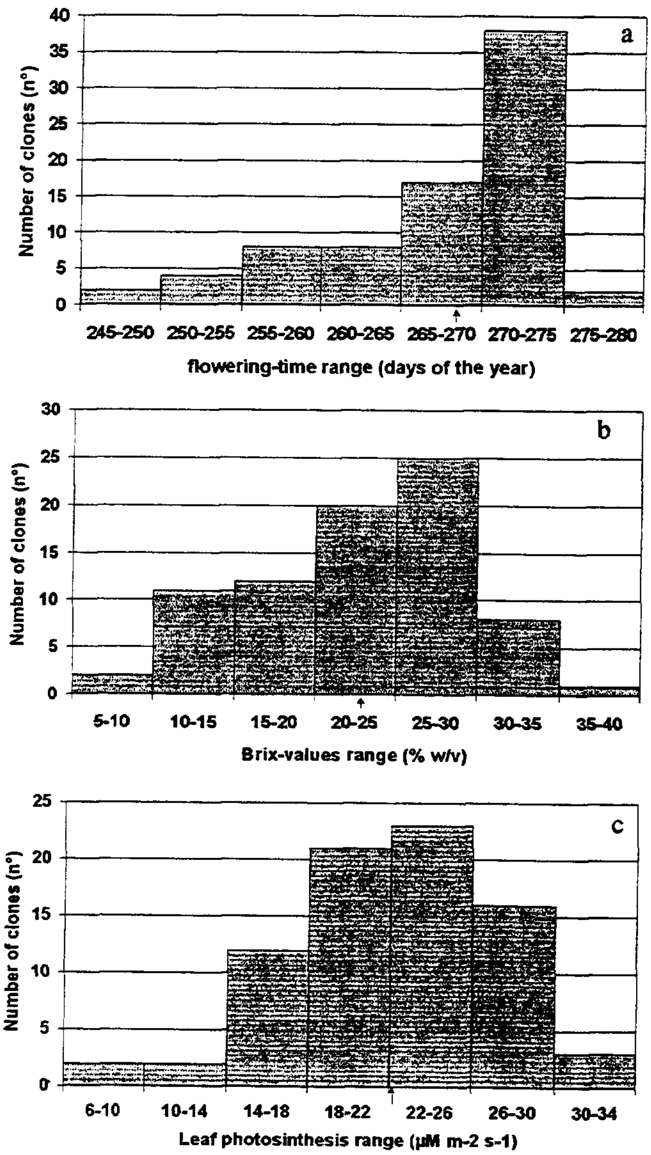

Figure 1 – Variability distribution of 79 Topinambur accessions for flowering time (a), upper leaves photosynthesis activity at flowering time (b) and for soluble sugars content (as brix values) into the stem at flowering time (c). Arrow indicates the mean of the populations.

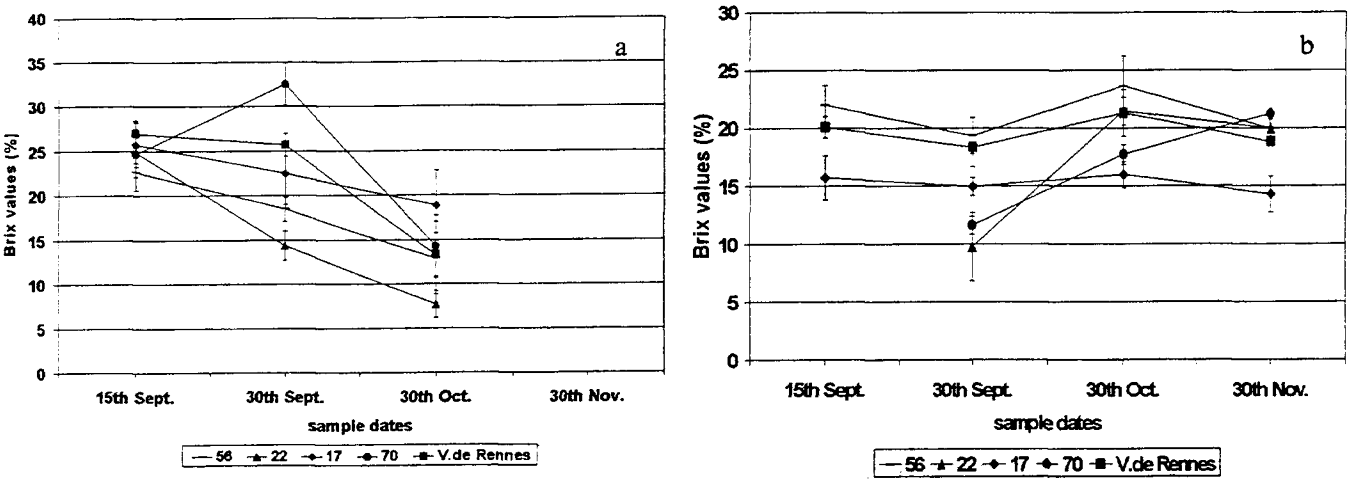

Figure 2 – Time course accumulation of soluble sugars (as brix values) into stems (a) and tubers (b) of 5 Topinambur accessions. 30[th] September and 30[th] November are harvest times for a stem and tuber crop, respectively. Bars represent Standard error.

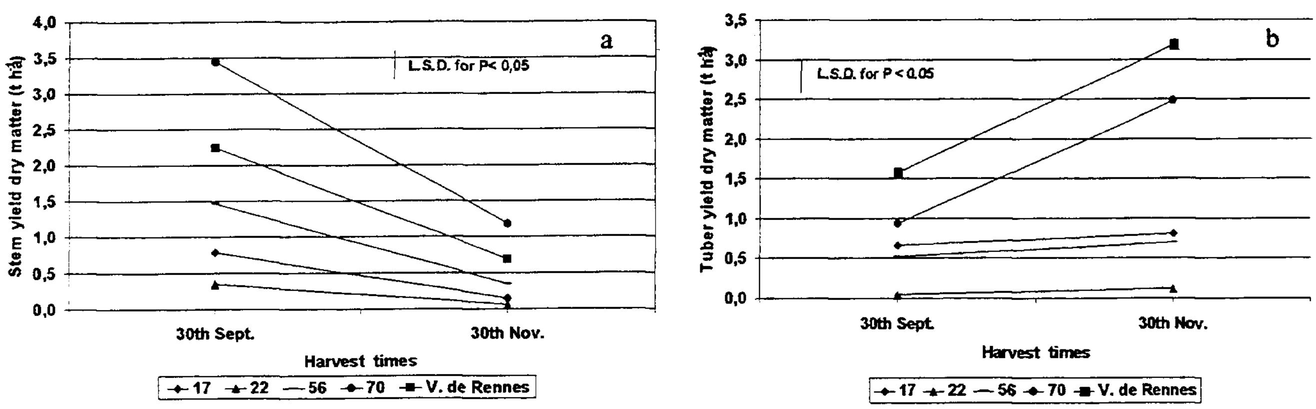

Figure 3 – Stem (a) and tuber (b) dry matter yield at two harvest times.