In vitro evaluation of tuberisation earliness and productive ability of new Italian potato clones

Agr. Med. Vol. 135, 180-187 (2005)

IN VITRO EVALUATION OF TUBERIZATION EARLINESS AND PRODUCTIVE ABILITY OF NEW ITALIAN POTATO CLONES

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SUMMARY - The earliness and productive ability represented by percentage of tuberized plant and number of tubers per plant of 12 new potato clones, an old Italian variety Viola Calabrese and two control cvs. Désirée and Spunta were evaluated *in vitro*. Tuberization was carried out on two media (Murashige and Skoog medium with and without CCC) and under two photoperiods (long day and short day). *In vitro* tuberization was significantly influenced by genotype, photoperiod and medium. Under inductive conditions (short day and CCC in the medium) the lower plant growth improved microtuber production achieving the highest Harvest Index (HI). Concerning earliness, two main groups were observed: a group in which tuberization resulted anticipated under short day, such as Désirée cv., and a group in which long day conditions were responsible for early tuberization such as Spunta cvs. MN 190 and ISCI 67, which had Spunta as parental type and showed similar behaviour to the reference cultivar for all factors studied.

Key words: harvest index, photoperiod, media culture.

INTRODUCTION

Field evaluation of new potato clones needs considerable resources in terms of space, time and work. *In vitro* tuberization seems to represent a valid and rapid alternative tool in evaluating the productive ability of new clones of potato (Vecchio *et al.*, 2002). Time and "degree of tuberization" observed in different cvs *in vitro* allowed them to be divided into maturity classes (Veramendi *et al.*, 2000). *In vitro* culture has already been considered a useful technique for gathering further information about the involvement of endogenous and exogenous factors in tuberization mechanisms (Duncan & Ewing, 1984; Ewing, 1990; Charles *et al.*, 1992; Kostantinova *et al.*, 1999). A short photoperiod encourages precocious tuberization to the detriment of leaf and stem growth and may be considered an inductive condition (Gregory, 1956; Wang and Hu, 1982; Garner & Blake, 1989). In other experimental trials, long photoperiods favoured tuberization or, at least, allowed an increase in tuber production with a greater development of leaf area and a longer growth period (Hussey & Stacey, 1981; Ezekiel & Bhargava, 1993). Moreover, Sladky & Bartosova (1990) observed that a long photoperiod encourages precocious tuberization in early varieties, while a short photoperiod accelerates it in late varieties.

The different response to photoperiod was also attributed to genotype effect (Dobránszki, 1996; Vecchio *et al.*, 1997), mother-plant age and cutting position at propagation time (Struik & Lommen, 1990).

Induction and tuberization levels are likewise affected by medium composition (Rossel *et al.*, 1987; Forti *et al.*, 1990), usually consisting of MS salts (Murashige & Skoog, 1962) modified by the addition of sucrose, with a tuberization concentration optimum ranging from 6-8% (Lawrence & Barker, 1963; Obata-Sasamoto & Suzuki, 1979; Wang & Hu, 1982). However sucrose cannot be

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considered the only stimulating substance (Ewing & Struik, 1992). Gibberellins play an important role in tuberization regulation, inhibiting or retarding the process (Pont Lezica, 1970; Tizio, 1971; Jackson *et al.*, 1997). Other growth regulators affecting tuberization are CCC ([2-cloroethyl] trimethylammonium chloride) with an antigibberellic effect and ETH (2-chloroethilphosphonic acid) utilised separately or together (Bryan *et al.*, 1992; Vecchio *et al.*, 1994). The positive effect of CCC on tuberization was not identical for all the genotypes belonging to the species *Solanum tuberosum* L., indicating that this compound has a genetic-specific effect (Harvey *et al.*, 1992; Andrenelli, 1996).

The aim of this study was to evaluate the *in vitro* tuberization ability, expressed as percentage of tuberized plant, number of tubers per plant and harvest index, of 12 new potato clones selected in Italy for the project "Miglioramento Genetico della Patata" of MiPAF (Ministero delle Politiche Agricole e Forestali).

MATERIAL AND METHODS

Plant material

The new clones were supplied by the following institutions: the "Istituto Sperimentale per le Colture Industriali" (ISCI) who bred ISCI 4052 [(*Agata* x *Jaerla*) x (*Cilena* x *Wn106-81*)], ISCI 67 [*Liseta* x (*Concorde* x *Wn 106-81*)] where *Liseta* parentage are *Spunta x SVP VE 66 295*, ISCI 83 [*Aurora* x (*Obelix* x *Wn 106-81*)], ISCI A9 (*Melissa* x *Felsina/3*), ISCI B31 (*Monalisa* x *Romina*) ISCI C60 (*Monalisa* x *92B-15*) and the "Consorzio Provinciale per la Valorizzazione delle Produzioni Agricole Mario Neri" (MN) who bred MN 190 (*Spunta* x *Ukama*), MN 270 (*Primura* x *Ausonia*), MN 274 (*Bryza* x *T 704*), MN 278 (*Kaptha* x *H5*), MN 284 (*Oldina* x *103 B4*), MN 289 (*Elvira* x *45 c1*). The two sets of clones were compared with two control varieties, Désirée, Spunta, and with an old Italian variety named Viola Calabrese.

Mother plants

These were obtained starting from meristematic cultures. The plantlets were multiplied by sub-culturing uninodal cuttings on growing medium consisting of MS salts, 30 g l^{-1} of sucrose and 2 g l^{-1} of phytagel (Sigma P-8169) as gelling compound. The cultures were placed in a climatic chamber under temperature conditions of 18 °C light-off and 24 °C light-on, light intensity of 70 µmol m^{-2} sec^{-1} and 16 h photoperiod for 30 days.

Tuberization

The ISCI and MN clones were separately evaluated under the following experimental conditions: two tuberizing media, modified MS (MS whole strength, 80 g l^{-1} of sucrose, 2 g l^{-1} of phytagel) with and without the addition of CCC (500 mg l^{-1}), respectively indicated as MSC and MS and two photoperiods, short day of 8 h light (SD) and long day of 16 h light (LD). The experimental unit was a 60 ml glass test tube containing 10 ml of medium and a single uninodal cutting. The experiment lasted 91 days and was performed in two climatic chambers under the same temperature regime and light intensity adopted for mother plant propagation.

Experimental design

The experimental design was a *split-plot* design with photoperiod as the main plot factor. The different treatments combinations were organised according to a randomised block experimental design with 3 replications, growing 4 vitroplants per treatment.

Statistical analysis

Weekly observations were made on tuber formation in order to determine the Tuberization Earliness (TE), expressed as the number of days necessary to form the first tuber.

Plant Dry Weight (PDW), Tuber Dry Weight (TDW) and Tuber Number per Plant (TNP) were recorded at the end of each trial, also calculating the Percentage of Tuberized Plants (PTP) and the Harvest Index (HI) as the ratio TDW/(TDW + PDW).

Quantitative variables (PDW, TDW, PTP, TNP and HI) were subjected to analysis of variance (split plot ANOVA, Bonferroni multiple pairwise comparison test), to detect significant differences ($P < 0.05$) among treatments and their interactions. PTP was previously transformed as $Y = \arcsin X^{1/2}$, where X represents PTP.

RESULTS

Percentage of tuberized plants

About 90% of plants tuberized under SD showing a difference of 20% compared to those grown under LD in ISCI clones (Fig. 1A). In MN clones the presence of CCC induced an increase in the number of plants with tubers (Fig. 1B). Genotypes MN 190, MN 289, MN 274 and Viola Calabrese surpassed the 80% of tuberized plants under SD, while MN 270 showed better performances under LD (Fig. 1C).

Tuberization earliness

The SD and CCC interaction effect significantly anticipated the formation of the first microtuber, while no difference occurred between media under LD (Fig. 2A). In particular, ISCI 83, ISCI C60, ISCI 4052, Viola Calabrese and Désirée anticipated tuberization under SD, whilst ISCI B31 and ISCI A9 tuberized earlier under LD. On the other hand, clone ISCI 67 and Spunta quickly tuberized under both photoperiods (Fig. 2B). The positive effect of SD on earliness was particularly evident in ISCI 83 and Viola Calabrese, in which tuberization was respectively anticipated by 24 and 25 days compared to LD conditions. In MN clones CCC caused significant precociousness of the first tuber formation (Fig. 2C). Tuberization was accelerated by SD, particularly in MN 274 and MN 289 (Fig. 2D). Considering the mean values, between SD and LD, the earliest clones were MN 270, MN 190 and MN 289 that respectively tuberized after 42, 45 and 46 days,

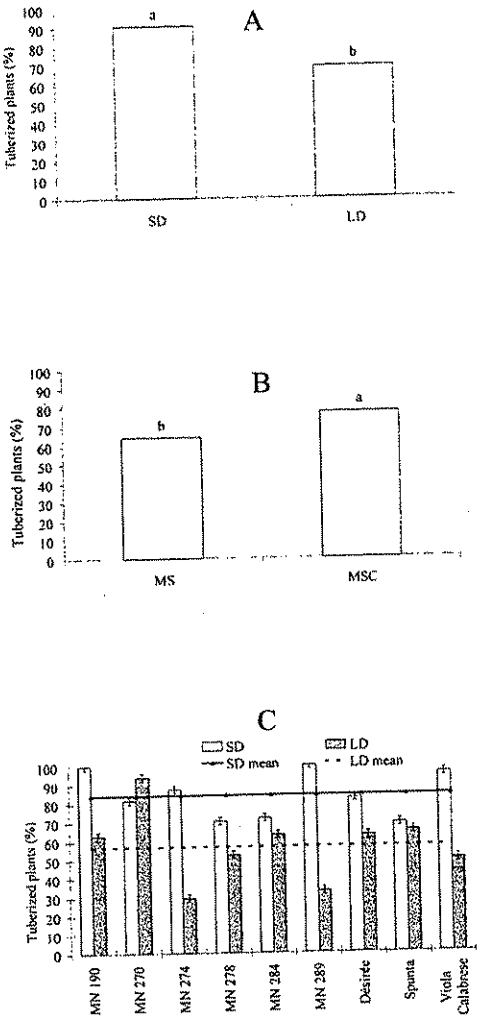

Fig. 1 - Means by interaction of photoperiod (A), medium (B) and photoperiod x medium (C) for percentage of tuberized plant (PTP). Different letters represent significant differences at 0.05 level according to Bonferroni's test. Vertical bars represent the standard error.

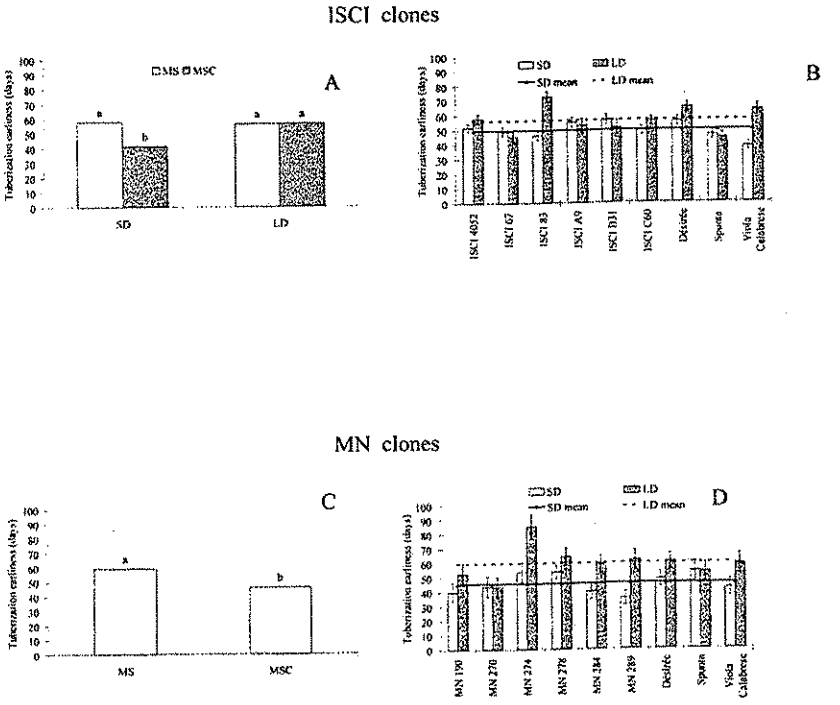

Fig. 2 - Means by interaction of photoperiod x medium (A), photoperiod x genotype (B and D) and medium (C) for tuberization earliness (TE). Different letters represent significant differences at 0.05 level according to Bonferroni's test. Vertical bars represent the standard error.

while MN 274 and MN 278 resulted the latest.

Number of tubers per plant

MS and MSC media under SD and LD respectively, induced about 2 microtubers per plant in clones of ISCI group (Tab. 1). The interaction clone x medium showed a different genotype behaviour: ISCI 67, ISCI B31, Spunta and Viola Calabrese displayed a better tuberization in the presence of CCC (Fig. 3A) and under LD (Fig. 3B); while ISCI 4052, ISCI 83, ISCI A9, ISCI C60 and Désirée showed better productive ability without CCC (Fig. 3A) and under SD (Fig. 3B). No significant difference arose among genotypes in the MN group (Tab. 1). Also for MN clones, the highest number of tubers was obtained under SD combined with MS. The growth regulator presence under LD increased tuberization, although not significantly.

Tab. 1 - Means by interaction of photoperiod x medium for tuber number per plant (TNP). Different letters represent significant differences at 0.05 level according to Bonferroni's test.

	ISCI clones		MN clones	
	MS	MSC	MS	MSC
SD	2.0 a	1.3 b	1.5 a	1.2 b
LD	1.3 b	1.8 a	1.2 b	1.3 ab

Harvest index

The presence of CCC induced higher values of HI in ISCI clones, except for ISCI

plex mechanism of tuberization. It is known that the tuberization process is controlled by a balance between endogenous gibberellins and an inductive stimulus, in which the former behaves as antagonist in the mechanism of *in vitro* tuberization (Hammes & Nel, 1975; Palmer & Barker, 1972). The positive effect of SD over tuberization is similar to that caused by antigibberellic agents (Kumar & Wareing, 1974). CCC exerts its effect over the hormonal endogenous balance modifying the ratio between growth and inductive hormones (Krauss & Marschner, 1982). These adjustments have different effects according to photoperiod used. In fact under LD conditions, during which growth hormones can reach the highest expression level, the antigibberellic action of CCC (Rademacher, 1999) is such that the level of these hormones is near to the threshold considered inductive for tuberization, while under SD conditions the modification induced by CCC over the GA complex is probably greater, and negatively interacting together with inductive endogenous conditions.

Finally, the possibility of *in vitro* evaluation of the productive ability of new clones was confirmed with field trials where the same genetic material was employed (Vecchio *et al.*, 2002). ISCI and MN clones showed different behaviour suggesting the possibility of their use in different potato cultivation typology (ware, early and extra-seasonal) in a Mediterranean area such as Italy.

ACKNOWLEDGEMENTS

The research was carried out within the project of MiPAF. The authors would like to thank the Istituto Sperimentale per le Colture Industriali of Bologna and the Consorzio Provinciale per la Valorizzazione delle Produzioni Agricole Mario Neri of Imola (Bologna). The Authors contributed equally to the work.

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Received: 09/12/04

Accepted: 04/04/05