EFFECTS OF THE PHYSIOLOGICAL AGE OF SEED POTATOES ON TUBER INITIATION AND STARCH AND DRY MATTER ACCUMULATION[1]

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ABSTRACT - The physiological age of seed potato tubers (*Solanum tuberosum* L.) modifies several morphological, physiological and agronomic characteristics of the crop. However, there is little evidence on the possible influence of physiological age upon the mechanism involved in tuber initiation and starch accumulation. This study investigated the effects of the physiological age of seed tubers on tuber initiation, starch synthase activity and dry matter accumulation and distribution. Physiologically aged seed potatoes promoted an early tuber initiation in the following crop. Early in the season the stolon tips of these plants showed an increase in starch synthase activity and an anticipated starch accumulation. During the rest of the season the starch synthase activity in the stolon tips of tubers grown from young seed was higher than those grown from the physiologically older ones, which decreased by day 70 after planting. Dry matter accumulation by leaves and stems did not differ between treatments, while tuber dry matter accumulation was higher in those plants originated from physiologically young tubers. These results confirmed that physiologically old seed anticipates tuber initiation and demonstrated that the pattern of starch synthase activity, starch accumulation and dry matter accumulation and distribution depends on the physiological age of seed tubers.

Index terms: *Solanum tuberosum*, stolon tips, starch accumulation, starch synthase, dry matter distribution.

EFEITO DA IDADE FISIOLÓGICA DAS BATATAS-SEMENTES NA INICIAÇÃO DA TUBERIZAÇÃO, NA AMIDO SINTASE E NA ACUMULAÇÃO DE MATÉRIA SECA

RESUMO - A idade fisiológica dos tubérculos-sementes de batata-inglesa (*Solanum tuberosum* L.) influi em algumas características da cultura seguinte. Não há, quase, evidências referentes à influência da idade dos tubérculos-sementes sobre a iniciação da tuberização e sobre a acumulação do amido. Com o objetivo de estudar essa influência, inclusive sobre a distribuição da matéria seca, realizou-se este trabalho. Batatas-sementes fisiologicamente velhas provocaram rápida iniciação da tuberização na cultura seguinte. No começo da estação, as pontas dos estolhos destas plantas mostraram crescimento do amido--sintase e uma antecipada acumulação de amido. Durante o resto da estação, o amido-sintase nas pontas do estolho dos tubérculos provindos de sementes jovens foi maior do que a dos provindos de sementes velhas. A acumulação de matéria seca em folhas e estames não diferiu entre os tratamentos; e em tubérculos, foi maior nas plantas provenientes de batatas-sementes jovens. Estes resultados confirmam que as batatas-sementes fisiologicamente velhas antecipam a iniciação da tuberização e mostram que o padrão da atividade do amido-sintase, a acumulação de amido e a acumulação de matéria seca dependem da idade fisiológica das batatas-sementes.

Termos para indexação: *Solanum tuberosum* L., pontas dos estolhos, acumulação de amido, distribução de matéria seca, amido-sintase.

INTRODUCTION

Since Van der Zaag (1973) pointed out the importance of crop and storage conditions that determine the physiological age of seed potatoes, much work has been carried out about the subject. Many reports have established the relationship between physiological age and some

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morphological, physiological and agronomic parameters; e.g.: sprouting, incubation period, crop growth, etc. (Caldiz et al., 1984, 1985; Bodlaender & Marinus, 1987; Caldiz, 1991). However, there is only limited information about the biochemical mechanisms regulated by the physiological age of seed tubers. Reust & Aerny (1985) determined that citric acid predominated in physiologically young tubers but it decreased as tuber aged, while malic acid increased. Caldiz et al. (1986) showed that at sprouting the total reducing sugar content is higher in physiologically old tubers while Van Es & Hartmans (1987) reported that also peroxidase activity is higher in physiologically old tubers. There are also other biochemicals parameters used as possible indicators of tuber age as reviewed by Van der Plas (1987). These previous results suggest that physiological ageing is related with higher metabolic activities during and after sprouting, probably associated with membrane integrity which is connected with senescence and decaying phenomena according to Van Es & Hartmans (1987) hypotheses.

It is also well known that the physiological age of the tuber influences dry matter accumulation and distribution (Kuisma, 1983; Bodlaender & Marinus, 1987; Caldiz, 1991). Moreover, physiologically old tubers may accelerate the phases between planting--emergence (Moll, 1985), emergence-tuber initiation (Caldiz et al., 1985) and emergence--senescence (Van der Zaag, 1973). The effects upon tuber initiation may be controlled by the internal hormonal balance (Tizio, 1979) and the action of enzymes, factors and cofactors related to starch synthesis (Claver, 1975) as it was, later, showed by Obata-Sasamoto & Suzuki (1979).

Nevertheless, at present there are no evidences about the influence of the physiological age between tuber initiation, starch accumulation and its relationship with dry matter accumulation and distribution. Hence the aim of this work was to study the effects of physiological age upon these parameters for a high sensitive cultivar to physiological ageing like Spunta.

MATERIALS AND METHODS

To obtain seed tubers of different physiological ages, potato tubers cv. Spunta were grown from mid-November to February in the Balcarce area (S.L. 37° 45'). The tubers were harvested in March and later stored in (a) a cold store at 4°C and in (b) a diffuse light store (natural daylight) at 17°C for 120 days. It is known that for Spunta cv. these conditions produce physiologically young and old tubers, respectively (Caldiz, 1991).

Physiological age

The physiological age of a seed tuber refers to the physiological stage inside its storage tissue at any time, and is a consequence of the environmental conditions during growing and storage (Caldiz et al., 1984, 1985; Caldiz, 1991). In this work the physiological age of the seed tubers was determined by the length of the incubation period, as follows. At the end of the storage period, samples of 25 tubers from each treatment (5 replications of 5 tubers each) were displayed in darkness at 21°C and 90-95% relative humidity, following Caldiz et al. (1984) procedures. The incubation period is the time elapsed since sprouting to the formation of new tubers upon these sprouts and the shorter the period, more advanced is the physiological age of the seed tuber.

Growing conditions

At the same time, at the beginning of November (South Hemisphere), seed tubers (40-60 g) from both treatments were planted in pots (15 liters) containing a loam soil fertilized with N-P-K (18-46-0) at a rate equivalent to 200 kg.ha^{-1}, in a randomized design with 5 replications. Hence, values obtained for all parameters came from these 5 replications. The soil was maintained at field capacity by periodical irrigation.

Since tuber initiation, periodical harvests were performed and each of the plants were separated into leaves, stems and tubers to determine dry matter accumulation and distribution. Plant parts were oven dried at 70°C till constant weight.

Soluble starch synthase activity

Enzyme extracts were prepared following Downton & Hawker's (1973) method as modified by Obata-Sasamoto & Suzuki (1979). Stolon tips harvested from five different plants (0.5-0.7 g fresh weight) were inmediately processed in the laboratory at 2-4°C. Samples were homogenized in 0.35 M Tris-acetate buffer, pH 8.5 containing 10 mM EDTA, 5 mM 2-mercaptoethanol (ME), 11 mM sodium diethyldithiocarbamate and 6% carbowax 4,000 (10 ml.g^{-1} fresh weight). Homogenates were centrifuged at 10,000 G for 20 minutes and the pellet was suspended in distilled water. A portion of this suspension was used for starch determination. The supernatant was taken to 25% with solid

$(NH_4)_2SO_4$, stirred for 1 hour and centrifuged at 10,000 G for 20 minutes. The precipitate was dissolved in 20 mM Tris-acetate buffer, pH 7.6 containing 10 mM EDTA and 5 mM ME and was dialyzed overnight with two changes of the same buffer. The dialyzed solution was used to measure the soluble starch synthase activity. For measurement of soluble protein content the dyalized solution was further dialyzed with three changes of 20 mM acetate buffer, pH 5.

Soluble starch synthase activity was assayed according to Tsay & Kuo (1980). The reaction mixture contained ADP (U $_{14}C$ 46,000 cpm per µl), EDTA (1 µmol), KCl (10 µmol) and tricine buffer, pH 8 (10 µmol) in a volume of 0.10 ml. For the assay, 50 µl of the extract was added to 50 µl of a 5% amylopectin solution. The reaction mixtures were incubated for 15 minutes at 37°C and stopped by adding 2 ml of 75% methanol-1% KCl solution. The starch was collected by centrifugation, washed twice with 2 ml of methanol-KCl solution and then dissolved in 1 ml water. The molar concentration of $_{14}C$-glucose formed was determined by counting an aliquot of 250 µl in 5 ml of dioxan containing 0.7% PPO, 0.03% dimethyl POPOP and 10% naphtalene with a Beckman LS 100-C scintillation counter.

Protein was determined according to the Lowry et al. (1951) method using bovine serum albumin as a standard.

Starch accumulation

The pellet fraction was centrifuged again. The precipitate was treated four times with 80% ethanol at 80°C for 15 minutes. Centrifugation was done at 6,000 G for 10 minutes. Starch was extracted from the residue with 52% perchloric acid, following the Mc Cready et al. (1950) technique. An aliquot of the starch extract was treated with 20% sodium chloride and iodine-potassium iodide; the solution was mixed and centrifuged at 6,000 G for 10 minutes. The precipitate was suspended in alcoholic sodium chloride, centrifuged and resuspended in alcoholic sodium hydroxide. Liberated starch was then centrifuged and washed with alcoholic sodium chloride as before. The precipitate was hydrolyzed with HCl 0.7 N at 2 atmospheres for 15 minutes, following the Pucher et al. (1948) method. Glucose was measured from a 1 ml aliquot of the hydrolyzate by the Cronin & Smith (1979) method.

For all parameters the results were analyzed by an analysis of variance and compared by Tukey's test (P: 0.05).

RESULTS AND DISCUSSION

Spunta is a very suitable cultivar for this kind of study, because is very sensitive to physiological ageing (Caldiz, 1991). Confirming previous results of Caldiz et al. (1984), the length of the incubation period was shorter for those seed tubers stored in diffuse light (38 days), resulting physiologically older than those stored in a cold store (57 days, significant difference according to Tukey: 5%). Hence, from now on, reference will be made to physiologically young or old tubers, the latter showing an advanced tuber initiation.

In physiologically old tubers the shorteness of the incubation period and the consequent advance in tuber initiation was associated with higher starch synthase activity and starch accumulation in the stolon tips of tubers derived from them (Fig. 1 and 2). Fifty days after planting, in these stolon tips, the starch synthase activity more than doubled that of stolon tips derived from physiologically young tubers. By day 60th these differences were still maintained. This suggests that the advance in tuber initiation of the physiologically old tubers is accompanied by an increased metabolic activity associated to those starch forming enzymes. This is in agreement with the finding that a higher amount of total reducing sugars occurred in physiologically old tubers (Caldiz et al.,1986), because this means that an important amount of substrate is available for starch synthesis which increases in stolon tips at time of tuber initiation (Hawker et al., 1979).

Later in the season, day 70 to 90th, both soluble starch synthase activity and starch accumulation showed a sharp decrease in those stolon tips coming from physiologically old tubers. Meanwhile, for the same period, enzyme activity and starch accumulation were higher in those stolon tips coming from physiologically young tubers (Fig. 1a and 1b). These differences may be attributed to the sink effect carried out by bigger tubers which in old plants could require an anticipated availability for photoassimilates. This sink-source relationship is well known in terms of dry matter mobilization from the haulms to the rapidly growing tubers (Moorby, 1970). Nevertheless, in this experiment the dry matter accumulation in the leaves and stems did not differ between treatments for most part of the growing season (Fig. 2a and 2b). Regarding to the dry matter accumulation in the tubers the situation was different, because a higher rate was found in those coming from physiologically young

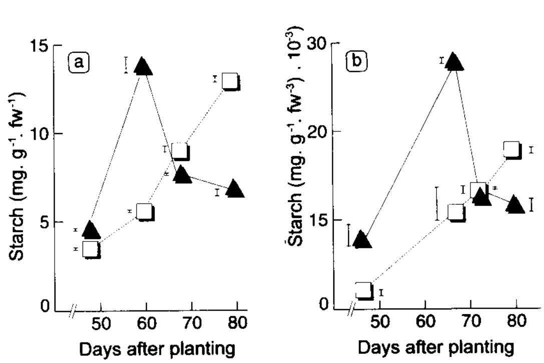

FIG. 1. Starch accumulation and starch synthase activity in stolon tips of potato tubers with different age. Young (□) and old (Δ) seed tubers. Vertical bars mean SE (2x).

seeds. At the final harvest tuber yield.plant^{-1} was also higher in these latter ones (Fig. 2c).

In accordance, in those plants coming from physiologically old seed tubers, dry matter distribution into leaves and stems followed the typical pattern proposed by Claver (1975) and Bodlaender & Marinus (1987), which found that physiologically old tubers produced higher foliage and stem growth earlier in the season, with young tubers producing similar results later (Fig. 3). Moreover, in this experiment tuber growth was higher, mainly by the end of the growing season in those plants coming from physiologically young tubers; these results are in agreement with Wurr (1978) and Kuisma (1983).

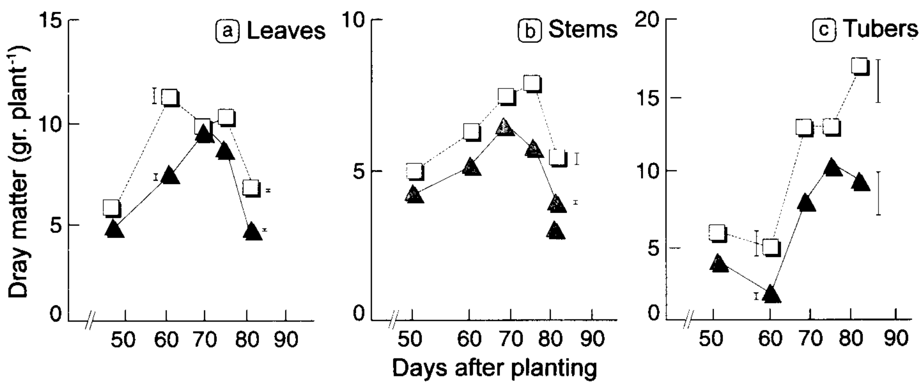

FIG. 2. Dry matter accumulation in the leaves, stems and tubers of plants with different age. Young (□) and old (Δ) seed tubers. Vertical bars mean SE (2x).

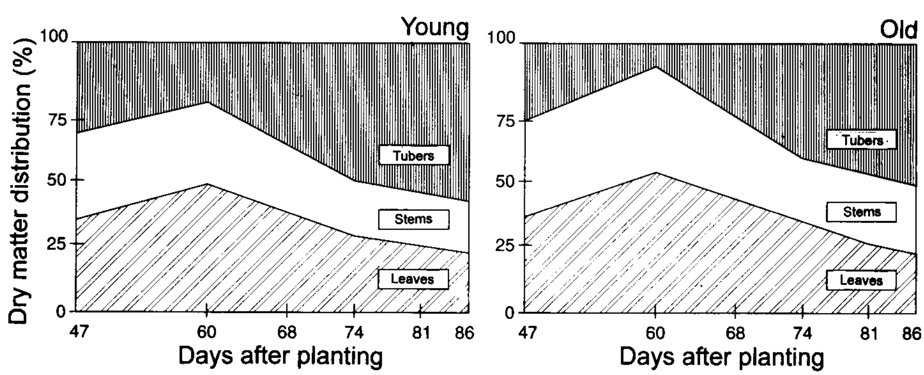

FIG. 3. Dry matter distribution within plants of different age for leaves, stems and tubers.

CONCLUSIONS

1. Physiologically old seed tubers advance the time to tuber initiation.
2. Physiological age modifies the pattern of soluble starch synthase actvity and starch accumulation.
3. Dry matter accumulation and distribution follow the typical pattern known for physiologically young and old tubers.
4. The physiological age of seed tubers modifies the biochemical, physiological and agronomic characteristics of the plants and tubers derived from them.

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