IN VITRO TUBERIZATION AND TUBER PROTEINS AS INDICATORS OF HEAT STRESS TOLERANCE IN POTATO

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Abstract

In vitro tuberization as a potential screening method for heat stress tolerance in potato, was assessed on nodal explants of Desirée, LT-2, Kennebec and Russet Burbank. Two tuber inducing media protocols were evaluated at 20 C and 28 or 30 C. Independently of the media protocol, heat stress significantly reduced tuberization. A delay in the formation of tuber initials was also observed in Desirée, Kennebec and LT-2 at 28 and 30 C compared to 20 C. Russet Burbank failed to tuberize under heat stress on both media. At higher temperatures Desirée either did not tuberize, or tuberized poorly on high sucrose-agar medium and tuberized the best of all cultivars, on low sucrose—Gelrite medium. Kennebec and LT-2 tuberized on both media. Medium with Gelrite gave better tuberization and more reproducible results than with agar. A high sucrose-agar medium, on the other hand, separated the heat tolerant clone LT-2 from the other cultivars.

Higher temperature reduced accumulation of patatin and 22 kDa protein in all cultivars. The reduction was greater in Kennebec and least in LT-2. The results indicate that microtuber production under heat stress conditions, combined with SDS-PAGE protein electrophoresis, can be considered as a preliminary method in screening potato germplasm for subtropical and tropical climates.

Compendio

Se ha ensayado la tuberización *in vitro* como un método potencial de tamizado para tolerancia al estrés por calor en papa, sobre explantas nodales de Desirée, LT-2, Kennebec y Russet Burbank. Se evaluaron dos medios de inducción de tuberización a 20 C y 28 ó 30 C. Independientemente del medio, el estrés por calor redujo significativamente la tuberización. Se observó también un retardo en la formación de iniciales del tubérculo en Desirée, Kennebec y LT-2 a 28 y 30 C comparado con 20 C. Russet Burbank no llegó a tuberizar bajo estrés por calor en ambos medios. Desirée no tuberizó o tuberizó muy poco en medio de agar con alto contenido de sucrosa y tuberizó mejor que todos los cultivares en medio Gerlita con bajo

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contenido de sucrosa. Kennebec y LT-2 tuberizaron en ambos medios. Ed medio con Gerlita dió mejor tuberización y resultados más reproducibles que el con agar. Por otra parte, un medio de agar con alto contenido de sucrosa diferenció al clon tolerante LT-2 de los otros cultivares.

Las temperaturas más altas reducen la acumulación de patatina y proteina 22 kDa en todos los cultivares. La reducción fué mayor en Kennebec y mucho menor en LT-2. Los resultados indican que la producción de microtubérculos bajo condiciones de estrés al calor, combinada con electroforesis de proteina SDS-PAGE, pueden ser considerados como un método preliminar para el tamizado de germoplasma de papa en climas tropicales y subtropicales.

Introduction

One of the major constraints of potato production in the subtropical and tropical climatic zones is limited heat stress tolerance in existing cultivars (4, 6, 13). For this reason germplasm selection for heat stress tolerance has been one of the principal goals in potato breeding programs.

Cultivar evaluation and/or screening for tuber initiation and bulking under heat stress has been conducted in the field or greenhouse (5, 6, 8, 9, 12, 13, 18, 20). This involves considerable amounts of time and space. Sattelmacher (18) refers to a "field evaluation bottleneck" to describe problems in screening for heat tolerance. A simple and effective means of screening genotypes for heat stress tolerance is a prerequisite for the production of suitable cultivars for subtropical and tropical regions. It is possible that tissue culture could provide a quicker method of screening larger numbers of clones for their capacity to tuberize and bulk under stress conditions.

The objective of this study was to examine the potential use of *in vitro* tuberization as a screening method for heat stress tolerance. Heat tolerant Desirée (9) and the CIP clone LT-2 (8); and heat sensitive Kennebec (1) and Russet Burbank (20) were used in this study. Two media were evaluated; one containing 8% sucrose, benzylaminopurine (BAP) and agar (11) and the other 6% sucrose, kinetin and Gelrite (2) as tuberization inducing and gelling agents, respectively.

Materials and Methods

(i) Plantlet Source and Micropropagation

Virus-free stock plantlets of Desirée were obtained from Fox Island Seed Potato Farm, Prince Edward Island, Canada. Kennebec and Russet Burbank were from the Plant Propagation Center, Fredericton, New Brunswick, Canada, and the CIP clone LT-2 was from our collection of potato germplasm. Micropropagation was done on potato nodal cutting medium (PNCM), as described earlier (19). Approximately 8 nodal explants with one bud per node were taken from each plantlet discarding apical and basal nodes and all the leaves. The explants were cultured in 25 × 200 mm

test tubes on 12 ml medium, one node per tube and grown under 160 μE m^{-2} s^{-1}, 400 - 700 nm fluorescent light (Photosynthetic Photon Flux Density, PPFD), 16 h photoperiod and 23/19 C day/night temperature.

(ii) In Vitro Tuberization

Experiment 1—Nodal explants were taken from six-week-old plantlets of all four cultivars as described above. Sixteen nodes were used per GA-7 Magenta vessel (Magenta Corp., IL.) containing 70 ml tuberization medium according to Machado and Shupe (11). The medium contained MS salts and vitamins, 8% sucrose, 2 g/l glycine, 10 mg/1 BAP and 6 g/l agar, pH 5.7. The vessels were wrapped in aluminum foil to occlude light and incubated at 20 or 30 C for eight weeks. Five vessel replicates were used per temperature for each cultivar. At weekly intervals the vessels were unwrapped and number of tubers recorded. After eight weeks minitubers were harvested and tuber number, diameter and fresh weight recorded.

Experiment 2—Conducted as *Experiment 1*, but with Desirée only. Eight-week-old plantlets were used as the explant source. The experiment was replicated seven times.

Experiment 3—Nodal cuttings were separated into apical (two nodal explants below the apical node), medial (four explants from middle stem) and basal (two explants above the basal node). Each vessel contained 4 apical, 8 medial and 4 basal nodes. Microtuber inducing medium was of Bourque *et al.* (2) and consisted of MS salts and vitams, 6% sucrose, 2.5 mg/1 kinetin and 2 g/1 Gelrite (Kelco Co., San Diego, CA.). Temperature treatments were 20 and 28 C. Other conditions were as in *Experiment 1.*

(iii) Protein Electrophoresis

Protein Extraction—120-150 mg samples of tuber tissue were homogenized with 3 vol of solubilizing buffer containing sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (3). The homogenates were centrifuged for 5 min at 13 000 $\times$ g in microfuge (Eppendorf). Supernatants were heated for 15 min at 80 C, cooled and centrifuged again.

Electrophoresis—35 μl aliquots of the protein extracts were applied on 12.5 $\times$ 13.5 $\times$ 0.1 cm, 10% SDS-polyacrylamide (PAGE) resolving slab gel with 6% stacking gel prepared according to Chua (3). 35 and 70 μl aliquots were used for the separation of protein extracted from 5 mm tubers obtained under heat stress. Conditions for electrophoresis and staining of the gels were as described by Chua (3).

Low range SDS-PAGE molecular mass standards were of Bio-Rad. The destained gels were equilibrated overnight against the solution composed of 7% acetic acid, 25% methanol and 2% glycerol in water and dried between two cellphane membrane sheets (Bio-Rad, Mississauga, Ont.) according to Wallevik and Jensenius (21). Molecular mass of tuber proteins

was estimated from a standard curve prepared by plotting logarithms of the molecular mass of the standards versus their electrophoretic mobility.

(iv) Dry Matter Determination

Half tubers of 5 mm in diameter harvested in *Experiment 3* were used for dry weight determination. The samples were dried at 60 C.

(v) Statistical Analysis

ANOVA and Duncan Multiple Range Test were performed using SAS computer program (SAS Institute Inc., Cary, NC.). Nonparametric data were transformed logarithmically for analysis.

Results and Discussion

In Vitro Tuberization—Figure 1 shows tuberization response to different temperatures. More shoot and root growth was observed at 28 and 30 C than at 20 C. The higher temperature also caused significant reduction of tuber induction and bulking after 8 weeks (Table 1). Tuber mass, total number of tubers and number of tubers ≥5 mm in diameter were significantly lower at 28 and 30 C than at 20 C. The results are consistent with findings reported from field trials (4, 6, 13) and greenhouse experiments with stem cuttings (5). The degree of response to heat stress varied however, between cultivars and tuberization media (Table 1). Russet Burbank failed to tuberize at higher temperature on both media. Tuber initials only were found after eight weeks of experiment on some shoots. Desirée either did not tuberize (*Experiment 1;* explants from 6 week plantlets) or tuberized poorly (*Experiment 2;* explants from 8 week plantlets) on Machado and Shupe's medium (11) but tuberized the best of all four cultivars at the higher temperature (*Experiment 3*) on the medium of Bourque, *et al.* (2). Kennebec and LT-2 tuberized on both media. On the medium with high osmotic potential (*Experiment 1;* 8% sucrose) LT-2 gave the highest tuber number and mass of all cultivars at 30 C (Table 1). The medium with Gelrite and lower sucrose (2) was more suitable for *in vitro* tuberization of Desirée, Kennebec and LT-2 at 20 C than the medium with agar and higher sucrose concentration (11). At 28 C tuberization on this medium was consistant with field performances reported for Desirée (9), LT-2 (8) and Russet Burbank (14) but not for Kennebec (1). Tuber initiation and bulking were the best in Desirée and Kennebec and the worst in Russet Burbank (Table 1, *Experiment 3*). Similar to the results of Bourque, *et al.* (2) obtained with Superior, Gelrite based medium gave more reproducible results, increased tuberization (particularly at higher temperature) and bulking (number of tubers ≥5 mm in diameter) (Table 1). For the selection of a heat tolerant gentoype, however, the high sucrose-agar medium was more suitable. It allowed to separate the heat stress tolerant clone LT-2 from the other cultivars (Table 1, *Experiment 1,* 30 C).

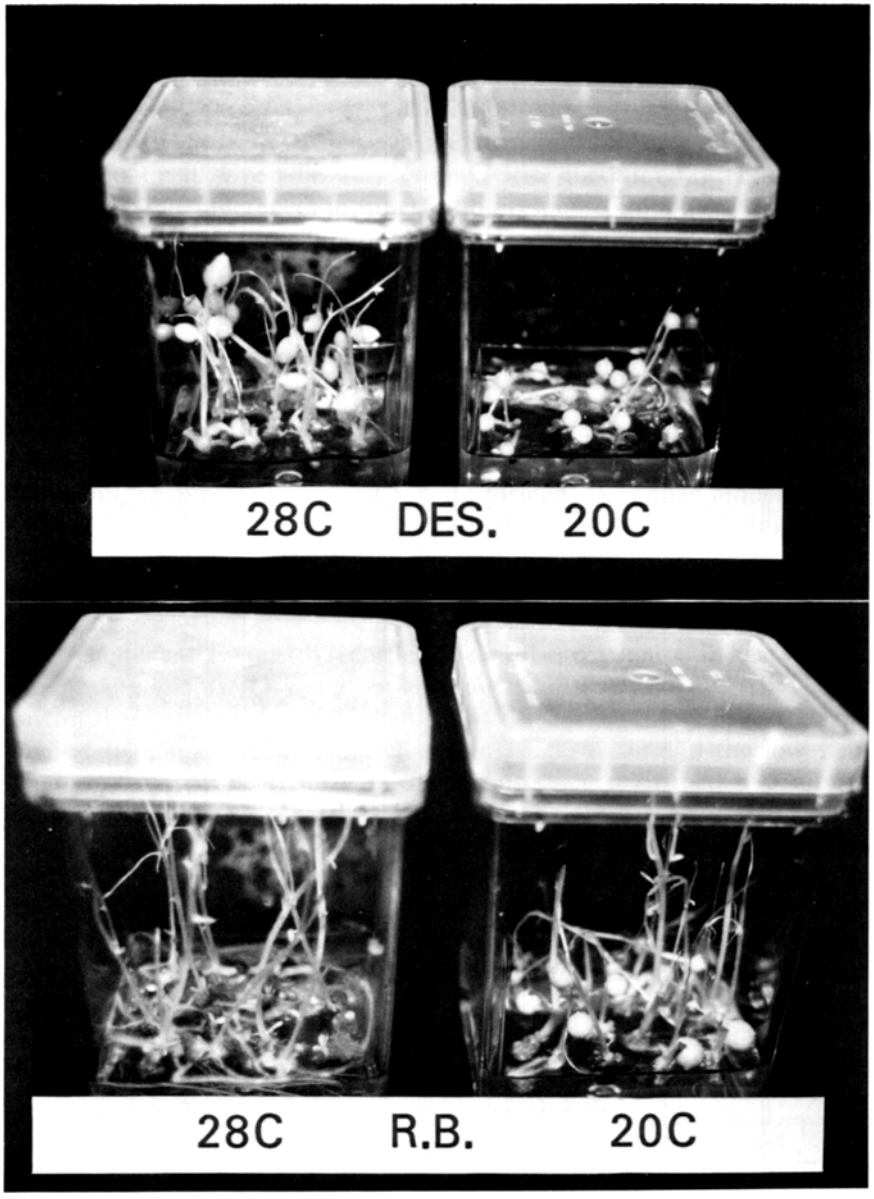

FIG. 1. *In vitro* tuberization of Desirée (DES.) and Russet Burbank (R.B.) at 28 and 20 C—*Experiment 3*, 6 week-old cultures.

TABLE 1. — *Temperature effect on* in vitro *tuberization of four potato cultivars on two media protocols*[1].

Cultivar	Tuber mass (g)[2] fresh weight		Tuber number[2] total		Tuber number[2] ≥ 5 mm	
	20 C	30 C	20 C	30 C	20 C	30 C
Experiment 1						
Desirée	.598** a	.000 b	12.0** a	0.0 b	2.0** a	0.0
Kennebec	.715** a	.024 ab	15.0** a	1.6 ab	3.9** a	0.0
LT-2	.544* a	.087 a	14.6** a	2.9 a	1.8ns a	0.0
Russet Burbank	.704** a	.000 b	13.4** a	0.0 b	3.6** a	0.0
Mean	.641**	.028	13.8**	1.8	2.8**	0.0
Experiment 2						
Desirée	.689**	.095	6.4*	2.6		
	20 C	28 C	20 C	28 C	20 C	28 C
Experiment 3						
Desirée	1.238 ns b	1.129 a	15.2 ns a	12.7 a	6.0* a	1.9 a
Kennebec	1.540* b	1.003 a	17.3** a	12.5 a	8.7* a	2.5 a
LT-2	2.040** a	.354 b	16.4** a	5.9 b	12.2** a	1.1 a
Russet Burbank	.621* c	.013 c	9.2** b	1.3 c	1.5ns b	0.0 b
Mean	1.360**	.625	14.5**	8.1	7.1**	1.4

[1]*Experiment 1* and *2*: Machado and Shupe (11) MS salts and vitamins, 10 mg/l BAP, 8% sucrose, 0.6% agar, pH 5.7; *Experiment 3*: Borque *et al.* (2) MS salts and vitamins, 2.5 mg/l kinetin, 6% sucrose, 0.2% Gelrite, pH 5.7. Results are expressed per plot consisting of 16 nodal explants.

[2]Means followed by the same letter in the same column within experiments are not significantly different at the 0.05 level of probability by Duncan's Multiple Range Test; a comparison of cultivar response.

*,**: Significant differences at 0.05 and 0.01 levels of probability for temperature as a variable.

When we examined the effect of the node position on a stem to the tuberization response, no significant differences were recorded (Table 2). In our experiment, however, shoot apex and the first apical and basal nodes were discarded.

Figure 2 illustrates a delay in the formation of tuber initials at 28 C compared to 20 C on lower sucrose-Gelrite medium (2). There were distinguishable differences in tuberization response among cultivars when expressed as percentage of total tuberization of each cultivar after 8 weeks. At 20 C, after 2 and 3 weeks respectively, Desirée tuberized 78.5 and 90.8%, Kennebec 51.3 and 79.3%, LT-2 47.5 and 80.1% and Russet Burbank 23.5 and 76.6%. At 28 C tuberization was not only delayed but also expanded in time. Tuberization after 2, 3 and 4 weeks was as follows: Desirée, 5.4, 63.1 and 81.1%; Kennebec, 8.3, 61.5 and 90.8%; LT-2, 0, 28.6 and 76.8%; and no tuberization in Russet Burbank. There was no significant increase in tuber initiation between week 3 to 8 at 20 C, and week 4 to 8 at 28 C when Duncan's Multiple Range Test was performed. A similar tendency was observed in the experiments with the higher sucrose-agar medium. The results indicate that 3 or 4 weeks tuberization time could be sufficient to evaluate cultivar response to heat stress if *in vitro* tuberization is used for screening.

Kheder and Ewing (8) recorded lower tuber dry matter in heat stressed greenhouse plants. Dry matter accumulation was also much lower in microtubers produced at 28 C than at 20 C (Table 3). Under heat stress the highest percentage of dry matter was in LT-2 (9.2%) and the lowest in Desirée (6.5%). Russet Burbank did not produce any tubers of 5 mm in diameter at 28 C.

Tuber Protein—Several authors reported 15 kilodaltons (kDa), 22 kDa and patatin (40 kDa) as the major storage proteins in tubers (10, 15, 16, 17) and microtubers (2). The protein bands with corresponding electrophoretic mobility could also be detected on SDS-PAGE electrophoregrams in our study (Figure 3). Hannapel, *et al.* (7) recorded reduction of the synthesis of tuber proteins after GA application. GA inhibited accumulation of patatin

TABLE 2. — *Effect of the node position on stem on tuberization response*[1].

	Tuber mass (mg)[2] fresh weight		Tuber number[2]	
Node position	20 C	28 C	20 C	28 C
Subapical	40 ± 23	23 ± 26	0.94 ± 0.26	0.59 ± 0.39
Medial	44 ± 21	17 ± 15	0.94 ± 0.22	0.50 ± 0.33
Basal	41 ± 22	21 ± 26	0.83 ± 0.41	0.50 ± 0.39

[1]Evaluated after 8 weeks of tuberization, *Experiment 3.* Results are means for all four cultivars expressed per node.

[2]F test did not indicate significant differences; ±, standard deviation.

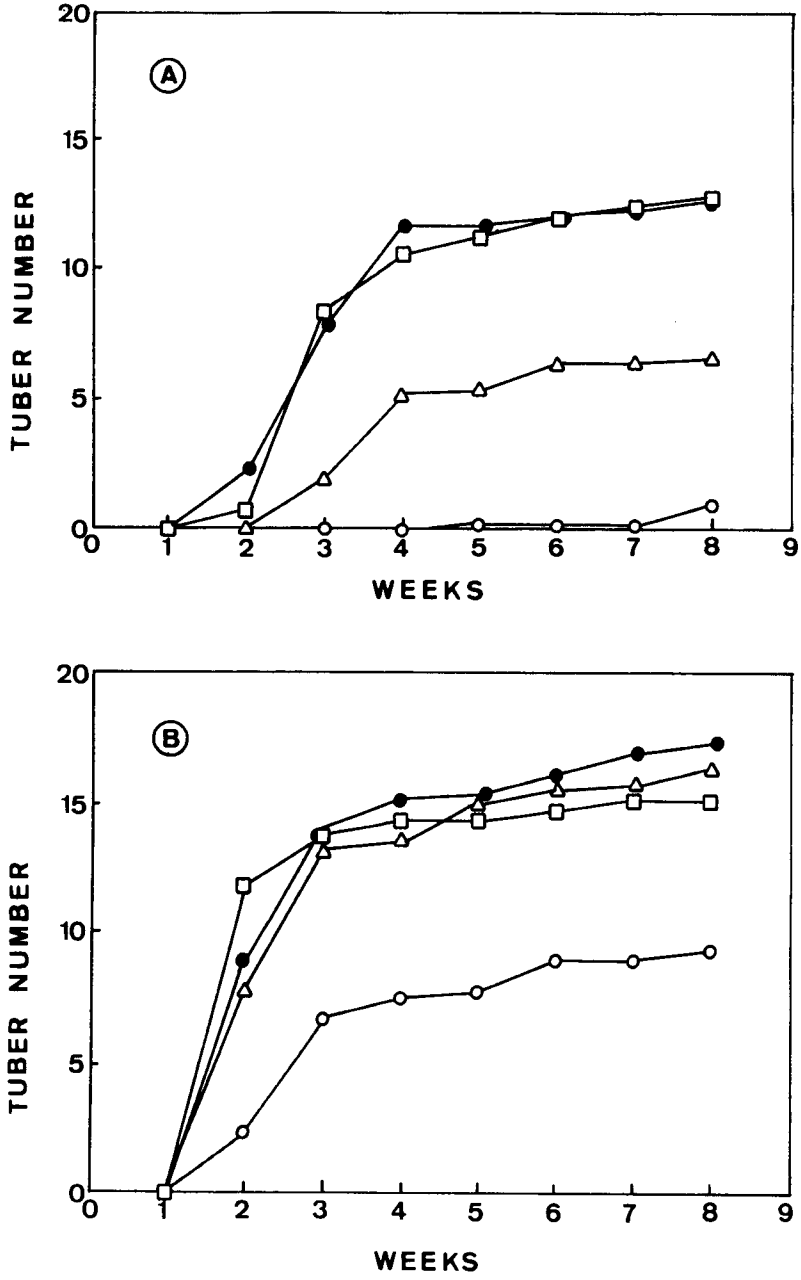

FIG. 2. Temperature effect on microtuber initiation on potato nodal explants. Cultures were incubated at 28 C (A) or 20 C (B)—*Experiment 3.* Results are means of five replicates of 16 explants each. Russet Burbank (0), Kennebec (●), Desirée (□), LT-2 (▲).

TABLE 3. — *Effect of tuberization temperature on dry matter content.*

Cultivar[1]	Temperature 20 C	28 C
	Dry matter (%)	
Desirée	10.6	6.5
Kennebec	9.9	7.1
LT-2	10.5	9.2
Mean	10.3	7.6

[1]Approximately 5 mm diameter microtubers, harvested after 8 week tuberization of nodal explants, were analyzed.

as well as 22 and 15 kDa proteins (7). Low patatin content was also found in non-induced stolons (14).

As *in vivo* (16), patatin, and 22 and 15 kDa proteins were demonstrated to be relevant to minituber bulking *in vitro* (2). In our study heat stress caused reduction of accumulation of patatin and 22 kDa protein in all three cultivars, tuberizing at 28 C (Figure 3). The changes were independent of tuberization medium. The 22 kDa protein seems to be an even better indicator of the cultivar response to heat stress than patatin. Accumulation of this protein was the most drastically reduced in Kennebec and the least affected was LT-2. In contrast to GA treatment (7), the 15 kDa protein was influenced very little by heat stress (Figure 3). These results also indicate that LT-2 and Desirée were less influenced by heat stress than Kennebec (Figure 3).

We suggest that *in vitro* production of microtubers, combined with the evaluation of accumulation of the major storage proteins, 22 kDa protein in particular, can be considered for preliminary screening of potato germplasm for heat stress.

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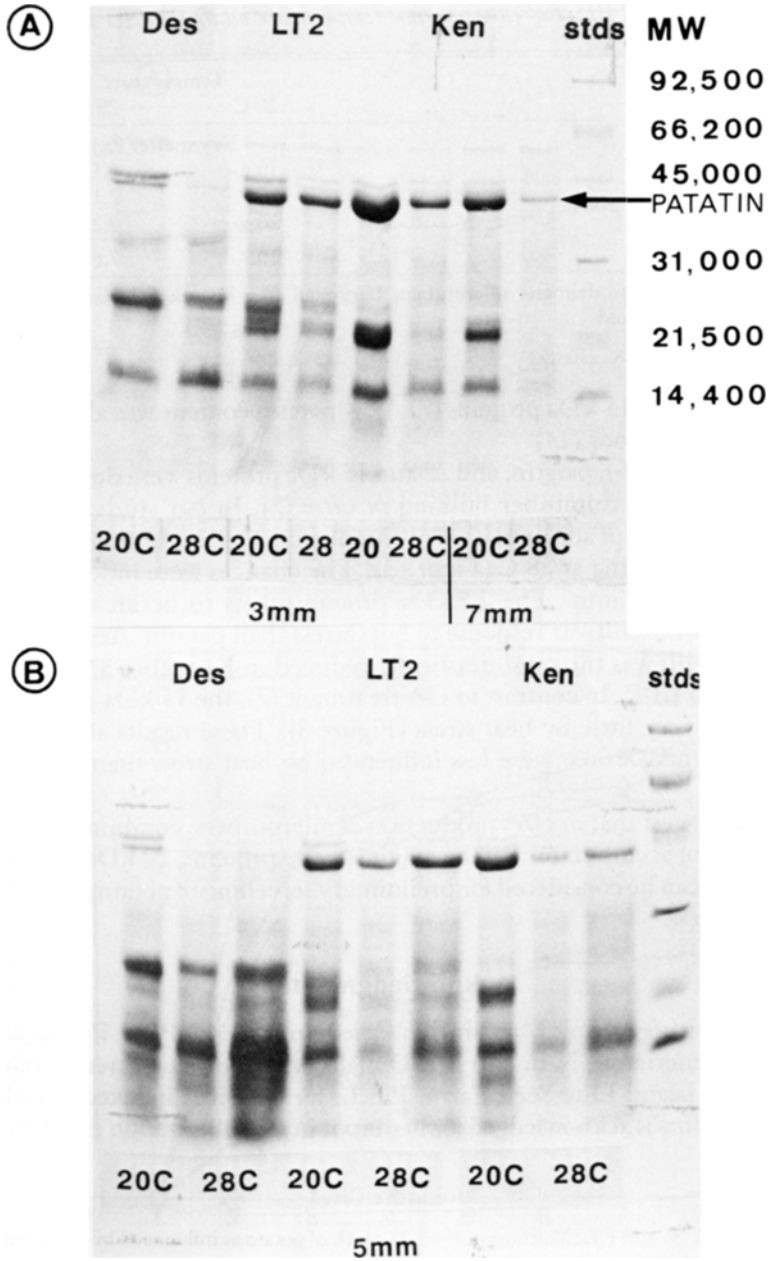

FIG. 3. SDS-PAGE electrophoregrams of total extractable protein from 3 and 7 mm microtubers (A) and 5 mm microtubers (B) obtained at 20 and 28 C in *Experiment 3.* Desirée (Des), LT-2 (LT-2), Kennebec (Ken) and molecular markeers (stds). Arrow indicates patatin bands. 35 μl aliquots of protein extracts were applied; in 5 mm tuber grade from 28 C, duplicate samples of 35 and 70 μl were used.

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