SCREENING POTATO (*SOLANUM*) SPECIES FOR MALE FERTILITY UNDER HEAT STRESS

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Abstract

Heat stress limits botanical seed production by inhibiting flowering, pollen production and pollen viability. Three accessions (PIs) of each of 23 diverse *Solanum* species were screened for stability of fertility parameters under heat stress. Seedlings were grown to flowering in temperate conditions (16-25 C), then treated with three weeks of heat stress of up to 45 C for 4-6 hours per day at midday with nighttime lows of 18 C, and compared to temperate-grown controls. A highly significant effect of species, temperature, and their interaction was detected for flowering. This means species were inherently different and heat had a general depressing effect, but the degree of that effect varied among species. While most species had little flowering in the hot house, *S. commersonii* and *microdontum* flowered significantly more there than in the temperate house. Of species which flowered in hot conditions, some shed no pollen and some had good shed of mostly dead pollen, but only *S. commersonii, jamesii, kurtzianum* and *megistacrolobum* had good flowering, pollen shed and viability. This work is expected to contribute to the study of the genetic and physiological bases of heat stress fertility, true potato seed (TPS) breeding, and possibly improvement of tomato production under heat stress.

Compendio

El estrés por calor inhibe la floración, la producción de polen y su viabilidad, limitando así la producción de semilla botánica. El mantenimiento de la fertilidad bajo temperatura alta fue evaluado en tres accesiones (PIs) de cada una de las 23 especies de *Solanum* utilizadas en el present estudio. Las plantas fueron mantenidas bajo condiciones temperadas (16 - 25 C) hasta su floración, luego fueron sometidas durante tres semanas a temperatura alta por 4-6 horas (hasta 45 C al mediodía y 18 C durante la noche) y comparadas con los controles mantenidos a temperatura temperada. El análisis estadistico demostró que la floración fue afectada significativamente por la especie, temperatura y la interacción de ambas. Esto significa que las especies fueron inherentemente differentes y que la alta temperatura tuvo en general un efecto depresivo, pero el grado de este

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efect varió entre las diferentes especies. Mientras que la mayoría de especies mostró escasa floración bajo alta temperatura, *S. commersonii* y *S. microdontum* florearon significativamente más que los respectivos controles a temperatura temperada. De las especies sometidas a alta temperatura, algunas no produjeron polen y otras produjeron polen mayormente no viable. Sólo *S. commersonii, S. kurtzianum* y *S. megistacrolobum* mostraron buena floración y produjeron polen viable. Con este trabajo se espera contribuir al estudio de la base genética y fisiológia de la fertilidad bajo altas temperaturas, mejoramiento de semilla botánica, y posiblemente el mejoramiento de la producción de tomate bajo altas temperaturas.

Introduction

Potato is an Important World Crop—Potato is the most important world vegetable, with great and rapidly growing economic and nutritional impact (14). Historically, the crop has been propagated clonally from tuber "seed" pieces. Since cultivated potatoes are heterozygous outcrosses which suffer from inbreeding depression, clonal propagation allows cultivation of uniform superior clones regardless of their breeding value. Tuberlings also have stronger sprouting and establish vines faster than seedlings, resulting in greater tuber yields.

True Potato Seed—The potato crop can also be grown practically from true potato seeds (TPS), especially in developing countries. TPS solves the problem of seed cost in regions that have a great need for the productivity potatoes can offer. They are less perishable, have much less bulk to transport, and do not harbor most of the systemic pathogens notorious for debilitating the potato crop. TPS usually can be produced in the area of cultivation at minimal cost by the farmer (10). Tropical regions have been judged to have the most potential for TPS utilization (15). However, hot climates still present a major obstacle, since both vegetative and sexual reproduction of potato is sensitive to heat stress. This work is focused on identifying exotic potato germplasm with fertility under heat stress. Such materials would be a resource for applying TPS production to hot climates.

Past Relevant Work—It has long been recognized that heat stress reduces flowering, viable pollen and seed production in potato (2, 4, 7, 9). Previous work has not specifically focused on heat fertility screening among exotic species, but some related screening for survival, growth, and tuber yield under heat stress has been done. Reynolds and Ewing (16) screened 59 species and found accessions of S. *berthaultii, bulbocastanum, chacoense, commersonii, demissum, fendleri, kurtzianum, microdontum* and *vernei* to exhibit less heat stress symptoms in shoot growth at 30-40 C. Midmore and Prange (13) found physiological parameters associated with heat tolerance in *S. acaule* and *circaeifolium*. Li (unpublished) found leaf tissue survived for longer periods of heat stress for accessions of *S. demissum, guerreroense, mochicense,*

microdontum and *morelliforme*. Jaworski *et al.* (8) reported the field performance of 85 *Solanum* species grown from TPS in Georgia. They found that certain accessions of *S. pinnatisectum, commersonii, kurtzianum, jamesii* and *polytrichon* were most desirable in terms of germination, vigor, ground cover, and tuberization. The compiled reports of various workers (5) suggest that heat tolerance clusters in accessions of *S. bulbocastanum, chacoense, microdontum, megistacrolobum* and *pinnatisectum*.

Other workers have studied aspects of heat fertility. Haynes *et al.* (6) examined the flowering and pollen quality of heat tolerant selections of a *phureja-stenotomum* (PHU-STN) population. One genotype was superior in a moderate heat stress (26/22 C) regime. They concluded that germplasm with both heat tolerance and heat fertility would expedite the use of TPS in hot climates. Arndt *et al.* (1) found that male fertility, bumblebee attraction, and resulting OP seed production of potential TPS breeding parents was strongly influenced by variation in heat stress over the growing season. The TPS breeding program at the International Potato Center (CIP) practices selection for flowering and pollen quality under heat stress among potential TPS breeding parents. This has resulted in considerable gains in hot environment TPS production (10).

Materials and Methods

Source of Materials—The materials used were seedling populations obtained from the US potato collection, the Inter-Regional Potato Introduction Project, NRSP-6, Sturgeon Bay, Wisconsin. These were tuber-bearing species of Sec. *Petota* from the genus *Solanum*. Representative accessions were chosen from species broadly dispersed among the taxonomic divisions of potato classification.

Glasshouse Testing—These experiments were conducted on potted seedling transplants in the glasshouse in summer (August) at Sturgeon Bay during 1992 and 1993. Twenty-three species were chosen as a representative sample of tuber-bearing *Solanum* species. Each species was represented by three accessions. Each accession was replicated three times by randomly distributing 9 seedlings to three experimental units of three seedlings each. Species names, accession numbers and species abbreviations for materials used are listed in Table 1. Treatments were applied at the onset of abundant flowering and lasted three weeks.

Treatments—Parameters limiting fertility were evaluated in two environments: "hot" and "temperate". The hot environment was accomplished by setting glasshouse vents to open at about 40 C, with a light application of whitewash, and no other artificial heating or cooling. The temperate environment was in an adjacent air-conditioned glasshouse set at 18 C. A normal application of whitewash was needed to keep temperatures down. For both environments, sunshine was the only lighting used (approx. 14 hr

TABLE 1.—*Identification of* Solanum *species accessions evaluated for flowering and pollen quality under heat stress.*

Species (*Solanum*...)	Accessions (PI...)	Abbreviation
acaule	472661, 473481, 500047	acl
canasense	265863, 310956, 473345	can
chacoense	197760, 275139, 320293	chc
commersonii	243503, 472842, 473411	cmm
demissum	160208, 230589, 498232	dms
fendleri	275156, 497998, 498004	fen
gourlayi	265579, 473062, 500049	grl
infundibuliforme	265867, 472894, 498351	ifd
jamesii	195190, 275262, 458425	jam
kurtzianum	472923, 472941, 498359	ktz
megistacrolobum	265873, 473133, 498383	mga
microdontum	218225, 473171, 500041	mcd
oplocense	435079, 473185, 473190	opl
papita	249929, 283102, 498033	pta
phureja[1]	225665, 225678, 225679	phu
pinnatisectum	184774, 275236, 347766	pnt
polytrichon	184770, 255547, 498039	plt
spegazzinii	205407, 472986, 500053	spg
stenotomum[1]	195204, 230512, 292110	stn
stoloniferum	205510, 283109, 498057	sto
tarijense	195206, 473243, 473336	tar
tuberosum[1]	281034, 347773, 243364	adg
verrucosum	161173, 275255, 498062	ver

[1]Cultivated species. Others are wild.

days). Plants were watered and fertilized similarly to plantings for seed propagation at the genebank.

Experimental Design 1992—In 1992 species were planted in the hot house in a completely random design with three replicates. One additional set of experimental units was planted in the temperate house for comparison. Seeds were planted May 12th. Temperatures were monitored for two weeks prior to initiating the experiment. Thermographs in two locations in each house were in close agreement. They indicated daily pre-treatment temperature cycles consisting of 16 C nighttime minima and midday maxima of 21-27 C (2-4 hrs duration). The temperate house had the same nighttime minima as the hot house and midday maxima of 21-24 C (1-2 hr duration). Vents in the hot house were closed on August 3rd, at which time all buds, flowers and spontaneous fruit were removed from plants in both environments. Pollen was collected at the end of each of the following three weeks. Hot house temperatures after initiation of the experiment reached

2-4 hr maxima of 40-43 C every day with the exception of days 5, 12, and 13 which reached 36 C.

Experimental Design 1993—The 1993 experiment employed exactly the same materials and facilities. Three-seedling experimental units were arranged in a randomized complete block design with two blocks in the hot house and one in the temperate house. Seeds were sown on May 15. Daily temperature cycles prior to initiation of treatments on August 23rd had nighttime minima at 18 C for both houses. The temperate house experienced a daily midday maximum of 22 C for 2-4 hr. The hot house experienced daily midday maxima of 32-35 C. On August 23rd, when flowering had generally become abundant, hot house vents were adjusted, resulting in midday maxima of 37-45 C (4-6 hr) each day for the following three weeks. Daily heat exposure was somewhat more severe and consistent in the 1993 experiment. Temperate house temperatures and hot house nighttime minima remained the same after initiation.

Evaluation Methods—The 1992 trial focused on pollen production. Pollen was collected by vibrating mature flowers with an electric buzzer. Pollen production (shed) was scored subjectively on a scale in which 0 = no pollen and 5 = abundant pollen. Pollen viability was assessed as percent stained pollen (PSP) of 500 grain samples stained with aceto-carmine (11). These evaluations were done on the pooled plants within each experimental unit after one, two and three weeks in the hot house and after three weeks in the temperate house. Pollen shed and PSP in the temperate house were quite uniform and high (Table 2), so differences among accessions in the hot house were analyzed without statistical comparisons to temperate performance.

The 1993 trial concentrated on flowering abundance, recognized as the first limiting factor in the 1992 trial. Data was collected only once, after three weeks. In 1993, flowering abundance (number of flowers produced during the heat treatment) was determined in addition to pollen quality. Species (and perhaps intraspecific) variation for flowering was expected, so the ANOVA for this experiment partitioned effects of species, temperature and their interaction by considering accessions within species to be replicates. The shed and PSP of accessions with significant hot house flowering were determined. Those which apparently produced considerable levels of viable pollen were intermated within their experimental unit. Styles were collected after three days and examined with fluorescent microscopy for pollen tube penetration (12).

Results and Discussion

The 1992 Experiment—Average hot house scores for pollen shed and viability were very similar for each of the three weekly samplings, so these were pooled for the statistical analysis.

TABLE 2.—Solanum *species accessions with best*[1] *pollen shed and viability characteristics*[2] *under heat stress in 1992 and corresponding temperate performance.*

		Hot		Temperate	
SPECIES	PI	SHED	VIAB	SHED	VIAB
acl	472661	0.3 a	26	5	76
mga	498383	0.3 a	11	4	84
opl	473185	0.3 a	5	4	50
opl	473190	0.3 a	8	5	90
phu	225665	0.3 a	19	4	50
plt	184770	0.3 a	14	5	90
stn	195204	0.3 a	1	5	99
sto	283109	0.3 a	2	3	50
tar	195206	0.3 a	12	5	91
ver	161173	0.3 a	22	3	70
acl	500047	0.7 ab	18	5	97
can	473345	0.7 ab	18	5	80
cmm	473411	0.7 ab	29	5	96
fen	275156	0.7 ab	50	4	86
grl	500049	0.7 ab	17	5	98
ifd	472894	0.7 ab	36	5	97
jam	275262	0.7 ab	36	5	96
ktz	472923	0.7 ab	26	5	70
mga	473133	0.7 ab	47	5	98
opl	435079	0.7 ab	30	5	80
plt	255547	0.7 ab	49	5	98
pta	249929	0.7 ab	23	5	98
spg	472986	0.7 ab	30	5	90
sto	498057	0.7 ab	20	5	60
tar	473243	0.7 ab	34	4	98
can	265863	1.0 ab	29	5	99
can	310956	1.0 ab	34	5	83
chc	197760	1.0 ab	28	5	100
dms	230589	1.0 ab	36	5	90
fen	497998	1.0 ab	56	4	71
ifd	265867	1.0 ab	44	5	70
ifd	498351	1.0 ab	68	5	100
ktz	498359	1.0 ab	44	3	56
mcd	500041	1.0 ab	33	5	70
plt	498039	1.0 ab	47	5	97
spg	205407	1.0 ab	31	4	80
spg	500053	1.0 ab	44	5	90
stn	292110	1.0 ab	20	5	70
chc	320293	1.3 bc	49	5	99
cmm	243503	1.3 bc	53	5	99
dms	160208	1.3 bc	70	5	99
dms	498232	1.3 bc	50	5	95
jam	458425	1.3 bc	45	5	100
jam	195190	1.3 bc	45	5	99
fen	498004	1.7 cd	63	4	84
chc	275139	2.3 d	43	5	98

[1]Accessions with no flowers or pollen shed in the hot house are not included.
[2]Species: see Table 1 for key to abbreviations. PI=accession number. SHED=pollen shed rating (0=none...5=abundant). Means followed by different letters are significantly different at p=0.05 (lsd=0.8). VIAB=pollen viability (percent acetocarmine stained pollen grains in a sample of 500).

Pollen Shed: (SHED) scores in the temperate house were uniform and high. Over 90% of accessions had scores of 4 or 5. In the hot house, shed for all accessions was poor. The roughly 10% of accessions with scores > 1.0, (average = 1.5) were from species *S. chacoense, demissum, jamesii* (two accessions each), and *commersonii, fendleri* (one accession each) (Table 2).

Pollen Viability: (PSP) scores in the temperate house were uniform and high, with an overall average of over 85%. Average PSP of accessions with shed in the hot house was 33%. The best 20% of accessions had PSP of at least 45%. Those species were: *S. fendleri* (3 accessions); *demissum, jamesii,* (2 accessions each); *chacoense, commersonii, infundibuliforme, megistacrolobum, polytrichon* (1 accession each) (Table 2).

The 1993 Experiment was modified to focus on flowering, the first limiting factor for seed production. The experiment was initiated at what was judged to be onset of vigorous overall flowering. This was apparently achieved, since the average number of flowers per experimental unit in the temperate house was 76, and 75 for both blocks in the hot house at initiation. When initial flowering was analyzed, the only significant effect was that of species. In other words, innate differences in flowering of species were evident at the onset of the experiment, but the environments at initiation were equivalent. ANOVA done on the number of flowers produced during three weeks of heat stress revealed significant differences between environments. The temperate house block average of 72 was greater than both hot house blocks (31 and 35), demonstrating a significant effect of temperature at p=0.05. As expected, species also had a significant effect (p=0.05), since some species flower less than others given normal growing conditions. Lsd was calculated to classify the means. The best accessions generally corresponded well to the best accessions in the 1992 evaluation. The interaction effect of species and blocks (of different temperatures) was highly significant (p=0.01). This indicates that species vary in their response to heat stress with respect to flowering.

Increased Flowering in Heat: comparisons of flowering in the temperate and hot house revealed some accessions which evidently flower more under heat stress. Four accessions exceeded the 95% confidence interval by having at least 40 more flowers per experimental unit in the hot environment. Two of these were *S. microdontum* and two were *S. commersonii* (Table 3—accessions marked with "*").

Pollen Viability: Of the 38 accessions which flowered in the hot house, 21 shed no pollen. Shed of the remaining 17 accessions was: poor (in 10), moderate (in 5), good (in 2). Five of these 17 accessions had 0% viable pollen according to aceto-carmine staining. Thus, only a few accessions combined significant flowering, shed and pollen stainability. These were two accessions each of *S. commersonii, jamesii* and *kurtzianum* (Table 3).

Pollen Potency: Styles from intra-experimental unit-pollinated accessions combining good flowering, shed, and PSP were examined microscopically.

TABLE 3.—Solanum *species accessions with best*[1] *flowering, pollen shed and viability characteristics*[2] *under heat stress in 1993 and corresponding temperate performance.*

		Hot			Temperate		
SPECIES	PI	FLOW	SHED	VIAB	FLOW	SHED	VIAB
dms	498232	0.5 a	M	0	22.5	G	100
chc	197760	1.0 a	P	0	0.0	-	—
phu	225678	1.5 a	N	—	12.5	M	25
tbr	347773	1.5 a	N	—	6.5	M	19
phu	225679	2.0 a	N	—	18.0	G	85
pnt	184774	2.0 a	N	—	3.0	G	50
stn	195204	2.0 a	N	—	0.0	-	—
tbr	243364	2.0 a	N	—	5.0	M	16
acl	472661	2.5 a	M	55	44.5	G	100
mga	265873	2.5 a	N	—	29.0	G	82
tbr	281034	3.0 a	N	—	13.0	N	—
grl	500049	3.5 a	N	—	13.5	G	100
ifd	498351	3.5 a	N	—	29.5	G	100
sto	498057	4.5 a	P	0	31.5	G	100
ktz	472941	5.0 a	M	0	1.0	G	100
cmm	472842	6.0 a	P	40	0.5	G	50
plt	498039	7.0 ab	N	—	27.5	G	100
spg	500053	10.0 ab	G	38	65.0	G	50
tar	195206	10.0 ab	N	—	2.5	G	50
fen	498004	10.5 ab	P	15	22.5	G	100
can	265863	11.0 ab	N	—	22.0	G	100
jam	195190	11.0 ab	P	58	6.5	M	95
mga	473133	12.0 ab	P	43(+)	19.0	G	100
fen	497998	13.0 ab	N	—	32.0	G	85
pta	498033	13.0 ab	N	—	56.5	G	100
plt	184770	13.5 ab	N	—	18.0	G	89
acl	500047	14.0 ab	N	—	5.5	G	100
chc	320293	15.0 ab	P	0	9.0	G	50
fen	275156	16.0 ab	N	—	17.0	G	100
ktz	498359	17.0 ab	P	48	23.5	G	68
jam	275262	24.5 ab	P	9(-)	21.0	M	95
plt	255547	26.0 ab	N	—	13.0	G	50
ktz	472923	27.0 ab	M	40(+)	53.0	G	100
mcd	473171	39.5 *bc	N	—	0.5	G	50
jam	458425	43.0 bcd	M	87	23.0	M	83
mcd	500041	65.0 * cdc	N	—	22.0	G	50
cmm	473411	98.0 * e	G	96	27.0	G	100
cmm	243503	206.0* f	P	46	24.5	G	100

[1]Accessions with zero flowers in the hot house are not included.

[2]See Table 1 for key to species abbreviations. PI=accession number. FLOW=number of flowers. Means followed by different letters are significantly different at p=0.05 (lsd=36). "*" indicates accessions for which the magnitude of increase in hot house flowering over temperate flowering is in the p<=0.05 range. SHED=pollen shed rating (*N*one, *P*oor, *M*oderate, *G*ood). VIAB=pollen viability (percent acetocarmine stained pollen grains in a sample of 500. (+)=particularly good *in vivo* pollen tube growth; (-)=particularly poor; other accessions with stainable pollen in the hot house had *in vivo* pollen tube growth similar to that of temperate house pollen.

Surprisingly, most of the temperate house controls had rather variable growth of pollen tubes through the style. This made it difficult to assess the effect of the hot environment. However, pollen tube growth in the hot house was not appreciably reduced except for *S. jamesii* PI 275262. This might be expected considering that heat reduced the PSP of this accession from 95% to 9%. *S. megistacrolobum* PI 473133 and *kurtzianum* PI 472923 had uniformly outstanding pollen tube growth in the hot house despite a 60% reduction in PSP, and a shed rating of "poor" in the former case. Thus, stylar examination provided useful information about relative pollen potency not revealed by shed or PSP.

Relationship of Heat Fertility to Vegetative Heat Tolerance: Species with good heat fertility parameters are also reputed to have vegetative heat tolerance (see Introduction). However some heat tolerant species had poor flowering and/or pollen quality under heat stress. Thus, selection for heat fertility is not an automatic by-product of selection for vegetative heat tolerance. It should be noted that the heat stress applied in this experiment was quite severe and was relieved at night, which differs from most previous tests of vegetative heat stress.

Conclusions and Future Work—Seed production in a hot environment is dependent on several factors: adequate flowering, development and shed of viable pollen, fertilization, and fruit and seed development. This experiment tested an array of *Solanum* species for all but the last two factors. Outstanding germplasm was identified and some insights into selection were gained. Sexually reproducing species are all expected to have abundant shed of stainable pollen, so little advantage would be expected from preselecting for these traits in a temperate environment. Although flowering under temperate conditions varies considerably, response to heat was not uniform. Thus, accessions of *S. commersonii* and *microdontum*, having moderate flowering in the temperate house, produced many more flowers in the hot house. The accessions with the best flowering did not necessarily have the best shed, and those with the best flowering, shed and PSP did not necessarily have the best pollen tube growth through the style. This indicates that these factors need to be screened for individually. Does any one species or accession combine all factors of heat fertility at the highest levels? If not, it will be desirable to artificially synthesize the optimal genetic combination before crossing to cultivated *S. tuberosum* breeding stocks.

A logical next step will be to focus screening on accessions within promising species, then on individuals within promising accessions. In this way, outstanding individuals may be selected as models for physiological and genetic studies, and be used for fixing high heat fertility in parental stocks for enhancement. The qualities of these selected materials also clearly need to be confirmed in field growing conditions with natural heat stress exposure. The ability of these qualities to result in the ultimate goal of abundant fruit and seed set also needs to be confirmed.

Heat fertility problems in tomatoes may also be relevant. Tomatoes are an important world crop, and have a close phylogenic and genomic similarity to potato (17). In tomatoes, a lack of good heat fertility is more serious, since edible yield depends on fruit production. High heat fertility is rare among tomato germplasm, and some of the accompanying effects (*e.g.* small fruit) are undesirable (3, 18). We might expect that genetic exchange between these crops will someday be routine. If so, the pursuit of heat fertility qualities in potato, perhaps unavailable in tomato germplasm, becomes even more attractive.

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