Effect of Propagule Type and Growing Environment on Antioxidant Activity and Total Phenolic Content in Potato Germplasm

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ABSTRACT

Wild potato species are maintained primarily as botanical seed populations, so tuber trait studies require conversion of germplasm to tuber form. Such tubers may be obtained from seedlings produced directly from botanical seed or from plants grown from tuber propagules (tuberlings). Since most wild species require short days for tuberization, it is not possible to generate field tubers of these species in most USA locations. Historically, tubers for research evaluations have been generated in the artificial conditions of the winter greenhouse. Since the potato crop is normally grown from tubers produced from tuberlings grown in the field, it is important to know how much results differ when evaluating tubers that were produced from seedlings grown in the greenhouse. We compared antioxidant activity and phenolic content of tubers generated in the greenhouse at Sturgeon Bay, WI, from seedlings, and tubers generated from both seedlings and tuberlings in the field at the Kula Experiment Station, Kula, Maui, HI. A 'mini-core' set of 75 PIs representing 25 wild and primitive cultivated species was used. Differences in means of propagule types and growing environments were significant but not large. Average amount of antioxidant activity and phenolic content of tubers from field-grown seedlings was higher than that of tubers from tuberlings. These values were also higher in field-grown tubers than in greenhouse-grown tubers. Relative performance was similar regardless of environment or propagule type, with some important exceptions. Tubers of *Solanum pinnatisectum* and *Solanum jamesii* were high regardless of treatment. In contrast, tubers of certain *Solanum microdontum*, *canasense*, *stenotomum* and *commersonii* (species much more amenable to breeding) exhibited high antioxidant levels when produced in the field from tuberlings, but not when produced from greenhouse-grown seedlings. Thus, some germplasm may not exhibit useful antioxidant potential when tubers are produced in artificial greenhouse conditions.

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ABBREVIATIONS AND DEFINITIONS: PI=Plant introduction, a population unit of germplasm; AOA=Antioxidant activity, measured as µg of trolox equivalents per gram tuber fresh weight; PHEN=Phenolic content, measured as µg chlorogenic acid equivalents per gram tuber fresh weight; HFS=tubers generated from Hawaii field seedlings; HFT=tubers generated from Hawaii field tuberlings; WGS=tubers generated from Wisconsin greenhouse seedlings

RESUMEN

Las especies de papa silvestre se mantienen principalmente como poblaciones de semilla botánica, de tal manera que los estudios de las características requieren la conversión del germoplasma a la forma de tubérculo. Dichos tubérculos se pueden obtener directamente de semilla botánica o de plantas provenientes de tuberculillos. Desde que la mayoría de especies silvestres requieren de días cortos para tuberizar, no es posible generar tubérculos de estas especies en la mayoría de lugares de EUA. Históricamente, los tubérculos para evaluaciones de investigación se han generado en condiciones artificiales de invernadero de invierno. Dado que el cultivo proviene de tubérculos producidos de tuberculillos crecidos en el campo, es importante saber cuanto difieren los resultados cuando se evalúan tubérculos producidos de plántulas crecidas en el invernadero. Hemos comparado la actividad antioxidante y el

contenido fenólico de los tubérculos generados en el invernadero de Sturgeon Bay, WI, de plántulas y tuberculillos generados de plántulas y tuberculillos en el campo de la Estación Experimental Kula, Kula, Maui, HI. Se usó un conjunto `mini-core´ de 75 PIs que representan 25 especies silvestres y cultivadas primitivas. Las diferencias en el promedio de tipos de propágulos y ambientes de crecimiento fueron significativas pero no amplias. La cantidad promedio de actividad antioxidante y contenido fenólico de las plántulas crecidas en el campo fue más alta que la de tubérculos provenientes de tuberculillos. El contenido de los tubérculos crecidos en el campo fue también más alto que el de los tubérculos formados en el invernadero. El comportamiento relativo fue similar sin tomar en cuenta el tipo de ambiente o de propágulos con algunas excepciones importantes. Los tubérculos de *Solanum pinnatisectum* y *Solanum jamesii* fueron altos sin tomar en cuenta el tratamiento. Al contrario, los tubérculos de ciertos *Solanum macrodontum*, *canasense*, *stenotomium* y *commersoni* (especies más dóciles al mejoramiento), exhibieron altos niveles de antioxidante cuando provinieron de tuberculillos pero no cuando se produjeron de plántulas crecidas en el invernadero. Así, algún germoplasma puede no exhibir su potencial antioxidante cuando los tubérculos son producidos es condiciones artificiales de invernadero.

INTRODUCTION

Numerous phytochemicals function as antioxidants by inhibiting oxidation during physiological body functions (Klein and Kurilich 2000). Antioxidants identified in fruits and vegetables include vitamins and polyphenols such as flavonoids and carotenoids. These compounds contribute to food and sensory properties (Aviram 2002; Gil et al. 2000). They are also reported to prevent oxidative damage to DNA and modulate cancer initiation and tumor progression by scavenging free radicals (Jayaprakasam et al. 2006). Eberhardt et al. (2000) reported that most of the antioxidant activity from fruits may be from phytochemicals other than ascorbic acid, and that natural antioxidants from fresh fruit could be more effective than a dietary supplement. They also reported that a combination of phenolic acids and flavonoid extract exhibited a strong inhibition of colon- and liver-cancer cell proliferation *in vitro*, in a dose-dependent manner. These plant compounds are believed to work additively and synergistically (Lui et al. 2002). Therefore, consumption of plant parts instead of individual compounds is the recommended method of increasing antioxidant capacity and functionality.

High consumption of fruits and vegetables has been associated with a lower incidence of degenerative diseases such as cancer (Michels et al. 2000). Phenolic compounds in fruits and vegetables exhibit an anti-inflammatory effect on diseased skin cells (Murakami et al. 2002) as well as antiproliferative and anticancer properties (Jayaprakasam et al. 2006; Kampa et al. 2004) However, according to Liebman (2005) the consideration of antioxidants as the ultimate protection from free radicals that cause disease is too simplistic. There is no convincing evidence that antioxidants confer cardiovascular benefits, for example, though the theory that they may have benefits is promising. It has also been suggested that genetic differences may alter the risk of disease, which may explain the inconsistencies in some research results.

Antioxidants in potato have been previously studied (Reyes et al. 2005). Chu et al. (2002) proposed a bioactive index (BI=$^1/_2$[AOA score + antiproliferative activity score]) to enable consumers to choose vegetables in accordance with their beneficial activities. According to their results, potato ranked eighth out of ten vegetables studied. However, to estimate the actual impact of these vegetables, one might factor in the annual per capita consumption. Doing so, potato ranks second, even with Chu et al.'s (2002) assumption of no antiproliferative value. There are, however, reports of antiproliferative value in potato (Friedman et al. 2005; Yang et al. 2006). The current and future nutriceutical value of potato should also reflect the fact that potato is more affordable than most other vegetables. So in addition to being a food that efficiently delivers calories and basic nutrients, potato may also be the crop of choice for increasing the population's intake of specific health-promoting phytochemicals to levels expected to significantly reduce human suffering and health care costs.

The short day requirement for tuberization of most potato species and the fact that such germplasm is maintained in genebanks as botanical seed populations typically cause evaluation for tuber traits of exotic germplasm to be done on tubers generated from seedlings grown in pots in the winter greenhouse. For some traits, such artificially produced tubers may not exhibit the genetic potential that would be evident when introgressed into a cultivar that will be produced from field-grown tuberlings. But evaluation of tubers from field-

grown tuberlings requires both an extra clonal generation and a suitable USA field location, since quarantine laws make import of numerous lots of foreign-grown field tubers impractical. The University of Hawaii Agricultural Experiment Station farm at Kula, Maui, was identified as a site having the needed attributes for generating field-grown tubers of wild species: short days with moderate temperatures and soil, infrastructure, and cooperators able to support potato cultivation. Therefore, the objective of this study was to investigate the effect of two propagule types (seedlings and tuberlings) and two growing environments (greenhouse and field) on AOA and PHEN in tubers of *Solanum* species.

MATERIALS AND METHODS

Plant Material

A 'mini-core' set of 75 PIs representing 22 wild and three primitive cultivated species, previously selected by staff at the US Potato Genebank, was used for this study (Bamberg 1995). This set was designed not only to represent broadly the taxonomic diversity in the genebank but also to favor species that can more readily be grown and introgressed with cultivars and encompass a diversity of stress and disease resistances. Analysis for AOA and PHEN was conducted on tuber extracts. Tuberlings were generated on seedlings grown in a greenhouse at the genebank in Wisconsin in 2003 (Figure 1). These tuberlings were sent to Hawaii and grown in the field in 2004 to produce HFT. A matching set of botanical seeds were also sent to Hawaii, germinated in a greenhouse at the University of Hawaii, Kula Station, and the seedlings were transplanted into the field to produce HFS. The seedlings and tuberlings in the field were placed under black plastic row cover with drip irrigation. Fertilizer was injected into the irrigation system at the rate of 7 g of 20-20-20 per plant, three times over the growing season. For WGS, seeds were germinated in 2004 in the greenhouse under vermiculite, and the seedlings were transplanted into 10 cm - 400ml clay pots filled with commercial soilless potting medium. The seedlings were watered as needed with 1 g/L 20-20-20 fertilizer with micronutrients. Supplementary lighting was provided with 400 W alternating sodium and metal halide lamps 2.5 m apart and 1.5 m above the benchtops for 16 h days, until plants began to flower. Temperatures were maintained at 22 C day and 13 C night until harvest.

Sample Extraction

All plants from each PI were harvested and tubers bulked. From each bulked PI three samples of fresh tubers, 5 g each, were randomly collected and shipped to College Station, TX, for analysis. The samples were placed in falcon tubes and 15 ml of HPLC-grade methanol added. The samples were homogenized with an IKA Ultra-Turrax Tissuemizer for 3 min. Tuber extract was centrifuged at 31,000 g for 20 min. and 1.5 ml of supernatant was collected into microcentrifuge tubes for analysis.

Determination of AOA

Total AOA in tuber extracts was estimated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method (Brand-Williams et al. 1995). A 150 µl aliquot was placed into a scintillation vial to which 2,850 µl of DPPH methanol solution was added, and the mixture was placed on a shaker for 15 min. The

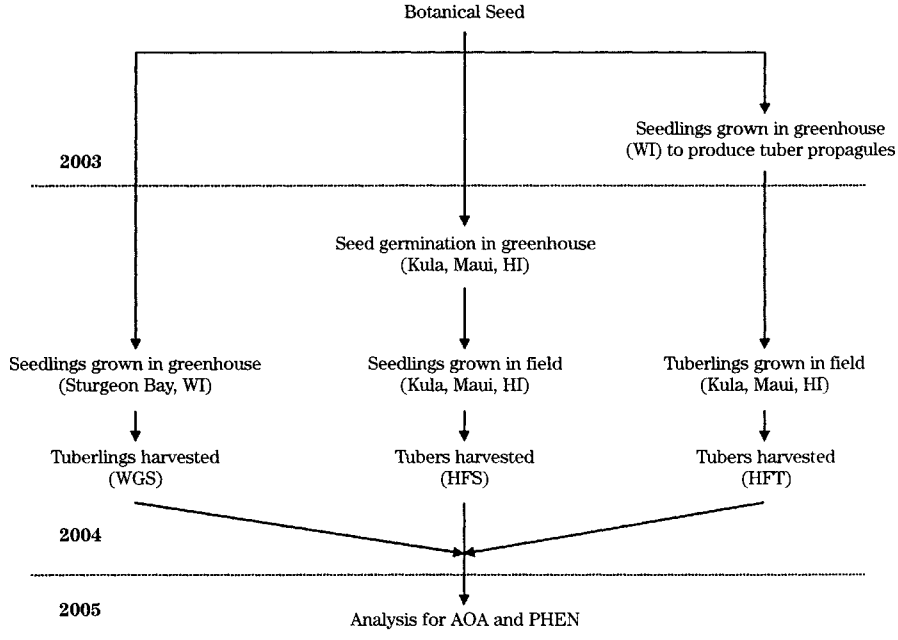

FIGURE 1.
Schematic illustration of the generation of potato tubers used for phytochemical analysis.

TABLE 1—*Comparison of antioxidant activity (AOA) and phenolic content (PHEN) in tubers of* Solanum *species produced on seedlings in a greenhouse at Sturgeon Bay, WI (WGS) and in a field at Kula, Maui, HI (HFS), and from tuberlings in a field at Kula, Maui, HI (HFS).**

		AOA			PHEN		
PI	Species	WGS	HFS	HFT	WGS	HFS	HFT
500047	*acaule*	161	585	369	626	1063	908
472661	*acaule*	532	515	236	1245	1172	929
243510	*bulbocastanum*	197	350	166	682	706	508
545751	*bulbocastanum*	171	207	106	586	647	458
310956	*canasense*	386	377	789	974	908	1503
265863	*canasense*	318	188	320	875	726	886
473345	*canasense*	114	129	183	657	779	733
197760	*chacoense*	380	145	137	1100	782	648
275139	*chacoense*	277	158	136	842	636	493
320293	*chacoense*	376	237	123	998	738	562
473411	*commersonii*	296	566	792	814	1102	1389
472837	*commersonii*	269	105	169	866	887	742
160208	*demissum*	168	197	400	694	665	1017
498004	*fendleri*	244	216	219	894	832	855
497998	*fendleri*	367	196	178	985	704	618
275156	*fendleri*	255	208	106	769	819	400
500049	*gourlayi*	201	139	200	810	629	861
473062	*gourlayi*	233	458	194	631	1153	901
265579	*gourlayi*	146	163	117	565	618	533
472894	*infundibuliforme*	212	159	113	751	677	640
275262	*jamesii*	594	734	591	1229	1289	1196
592422	*jamesii*	331	525	528	985	1284	1242
498359	*kurtzianum*	179	360	260	720	1035	823
472941	*kurtzianum*	197	431	248	746	954	678
498383	*megistacrolobum*	660	226	361	1314	983	944
265873	*megistacrolobum*	155	812	172	634	1357	607
473133	*megistacrolobum*	112	213	93	757	977	612
473166	*microdontum*	179	787	762	749	1439	1470
500041	*microdontum*	302	285	264	994	894	956
473171	*microdontum*	140	310	262	537	916	734
458367	*okadae*	700	581	159	1229	1241	684
498130	*okadae*	139	103	114	693	615	659
473190	*oplocense*	211	154	293	767	655	860
473185	*oplocense*	139	158	165	668	803	702
249929	*papita*	116	224	191	609	724	758
498033	*papita*	106	103	119	614	578	563
545725	*papita*	155	113	102	722	706	649
225679	*phureja*	101	348	519	823	1335	1200
225665	*phureja*	138	237	317	960	1237	1175
184774	*pinnantisectum*	892	899	880	1579	1700	1595
275236	*pinnantisectum*	766	844	834	1397	1576	1492
184770	*polytrichon*	133	357	208	565	1046	757
498039	*polytrichon*	208	161	158	793	663	636
255547	*polytrichon*	84	50	46	516	382	411
205407	*spegazzinii*	227	195	231	748	738	784
472978	*spegazzinii*	123	216	93	640	902	619
500053	*spegazzinii*	35	109	70	393	752	433
292110	*stenotomum*	427	362	757	987	1219	1449
195204	*stenotomum*	174	85	160	739	732	716
230512	*stenotomum*	128	250	107	717	892	1056
283109	*stoloniferum*	668	620	511	1234	1143	1022
498057	*stoloniferum*	172	205	420	712	839	1113
205510	*stoloniferum*	513	311	328	1144	1002	1022
473336	*tarijense*	355	302	468	1027	853	1135
473243	*tarijense*	556	101	218	1102	592	672

TABLE 1—*Continued.*

PI	Species	AOA			PHEN		
		WGS	HFS	HFT	WGS	HFS	HFT
195206	*tarijense*	122	142	191	587	590	782
243364	*tuberosum*	96	160	145	521	662	642
281034	*tuberosum*	69	141	83	707	852	1033
498062	*verrucosum*	124	467	414	575	1090	1021
Means		268	305	286	827	907	856
LSD[1]		78	48	63	112	118	106
LSD (WGS vs. HFS)			8			15	
LSD (HFS vs. HFT)			7			15	

*Unit for AOA values is μg TE/ gfw and for PHEN it is μg CE/gfw.
[1]Indicates least significant difference (LSD) at $P < 0.05$ for values within columns.

mixture was transferred to UV-cuvettes and absorbance recorded using a spectrophotometer at 515 nm. Trolox was used as a standard, and total AOA was expressed as micrograms of Trolox equivalents per gram of tuber fresh weight (μg TE/gfw).

Determination of PHEN

Total phenolic content was determined following the method of Singleton et al. (1999). First, 150 μl of sample extract was pipetted into scintillation vials, followed by an addition of 2.4 ml of nanopure water. Next, 150 μl of 0.25 N Folin-Ciocalteu reagent was added to each vial and allowed to react for about 3 min, and then 0.3 ml of 1n Na_2CO_3 reagent was added and left to react for 2 h. The spectrophotometer was zeroed with a blank (0.150 ml Methanol, 2.4 ml H_2O, 150 μl of 0.25 N Folin-Ciocalteu reagent, and 0.3 ml 1N Na_2CO_2) before sample analysis. Absorbance of sample extracts was read at 725 nm. Chlorogenic acid was used as a standard, and PHEN expressed as micrograms of chlorogenic acid equivalents per gram of tuber fresh weight (μg CE/gfw).

Statistical Analysis

An analysis was conducted to compare the effect of the two environments (greenhouse and field) on tubers grown from seedlings and the effect of propagule type (seedlings and tuberlings) on tubers grown in the field. The data for each comparison were analyzed separately using analysis of variance (ANOVA). A combined ANOVA for locations/environments sharing the same seed type was performed. PI, location, and PI-by-location interaction effects were determined using the general linear model (GLM) of the SAS version 9.0 software (SAS 2002), considering all variables as fixed effects. The importance of propagule type, growing environment, accession (PI), and the interactions of PI with propagule type and PI with environment on AOA and PHEN was determined by estimating the proportion of total variation attributed to each of them.

RESULTS AND DISCUSSION

Overall Variation

A wide and significant variation in AOA and PHEN was observed among PIs (Table 1). The highest (*S. pinnantisectum*) and lowest (*S. spegazzinii* and *S. polytrichon*) PIs were the same whether in greenhouse or field environments. The highest AOA value for PIs grown in the greenhouse was 892, exhibited by *S. pinnantisectum* (PI 184774), and the lowest value was 35 by *S. spegazzinii* (PI 500053). The highest AOA value in the field was 899, again by *S. pinnantisectum* (PI 184774) and the lowest value was 46 in *S. polytrichon* (PI 255547). For PHEN, the highest value among greenhouse-grown PIs was 1579 for *S. pinnantisectum* (PI 184774), and the lowest value was 393 exhibited by *S. spegazzinii* (PI 500053). The highest value for PHEN in field-grown tubers was 1700 in *S. pinnantisectum* (PI 184774), and the lowest was *S. polytrichon* (PI 255547) at 382.

Treatment Means

WGS vs. HFS: The effect of environment was significant for tubers produced on seedlings, with WGS<HFS for both AOA and PHEN. Average values from the field (HFS) were only 11% greater than from the greenhouse (WGS) for PHEN (907 vs. 827) and 14% greater for AOA (305 vs. 268). However, some PIs exhibited more activity in the greenhouse than in the field. Table 2 confirms that a minor part of overall variation

TABLE 2—*Analysis of variance for antioxidant activity (AOA) and phenolic content (PHEN) in* Solanum *species grown in two environments (greenhouse and field), and from two propagule types (seedlings and tuberlings).*

Source	DF	AOA MS	AOA % variance¥	PHEN MS	PHEN % variance¥
			Propagule Type‡		
Rep	2	5377		42361	
Propagule type	1	29856**	0.01	230597**	0.01
PI	58	231570**	0.81	418641**	0.81
PI x Propagule	58	41723**	0.15	74180**	0.14
Error	234	1203		4895	
			Environment‡		
Environment	1	117339**	0.01	562965**	0.02
Rep	2	6195		45924	
PI	58	187266**	0.74	308484**	0.71
PI x Environment	58	56429**	0.22	96538**	0.22
Error	234	1619		5060	

¥Percentages of total variation attributed to each source of variance.
*Indicates significance at *P*-value< 0.05; ** Indicates significance at *P*-value< 0.01.
‡Propagule type analysis was done on HFS and HFT data; Environment analysis was done on WGS and HFS data.

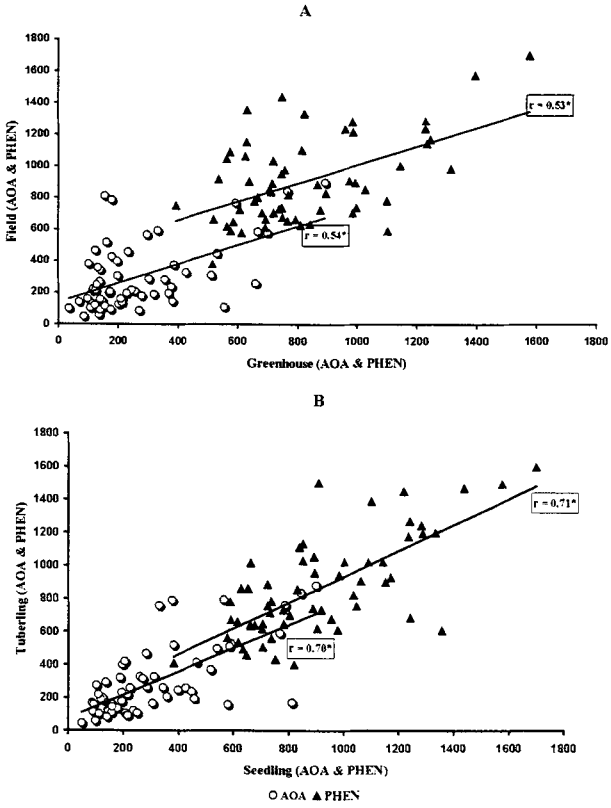

FIGURE 2.
Correlation of results—
(A) Greenhouse-grown versus field-grown tubers derived from seedlings (WGS vs. HFS). (B) Seedling-derived versus tuberling-derived tubers grown in the field (HFS vs. HFT).
*** Indicates that the correlation coefficients are significant at *P*-value < 0.05.**

was due to environment alone. Although these higher levels for field-grown tubers are not striking, previous work that examined the antioxidant levels of greenhouse-grown tubers of exotic species may have somewhat underestimated their potential as compared to field-grown cultivated stocks.

HFS vs. HFT: The effect of propagule type for field-grown tubers was significant, with HFS>HFT for both AOA ($LSD_{0.05}$ = 11) and PHEN ($LSD_{0.05}$ = 10). However, field-grown tubers from seedlings were only 6% greater for PHEN (907 vs. 856) and 7% greater (305 vs. 286) for AOA (Table 1). Table 2 confirms that only a minor part of overall variation was due to propagule type.

PI and Species Differences

Very large and significant differences were noted among PIs within treatments. These differences greatly exceed the $LSD_{0.05}$ values given in Table 1, and Table 2 shows that a large part (>70%) of overall variance is accounted for by PI for both AOA and PHEN. The best performing PIs for both AOA and PHEN irrespective of propagule type or growing environment were 184774 and 275236, belonging to *S. pinnatisectum*. PIs 473411 (*S. commersonii*), 310956 (*S. canasense*), 473166 (*S. microdontum*) and 292110 (*S. stenotomum*) exhibited more than 700 µg TE/gfw and 1300 µg CE/gfw for AOA and PHEN, respectively, in HFT, while 473166 (*S. microdontum*), 275265 (*S. jamesii*), and 265873 (*S. megistacrolobum*) were among the highest in HFS (Table 1). Results observed in this study agree with those reported by Hale (2003), in which the same high-performing PIs were observed. Most species were higher in AOA than *S. tuberosum* (PIs 243364 and 281034). These results direct attention to species that might be used to breed for high AOA and PHEN.

Correlations between Environments and between Propagule Types

An overall correlation of >50% was observed between WGS and HFS for both AOA and PHEN (Figure 2A), suggesting that, in general, rankings of PIs were quite similar regardless of environment. Similarly, a correlation of >70% was observed between HFS and HFT for AOA and PHEN (Figure 2B), suggesting that, in general, rankings of PIs of field-grown tubers were similar regardless of propagule type used.

For broad screening, it is helpful if the relative performance of PIs is generally similar regardless of the particular testing regime. Broad screening is usually limited by the great scope of germplasm that could be tested, so it is useful to have a simple, even if somewhat artificial, testing method that will narrow down that scope, perhaps to the best species. When identifying particularly outstanding parents for breeding, however, it is important to know that the testing regime used identifies the best material under practical crop production conditions. In this study, a significant part of overall variation was accounted for by both interaction of PI with propagule type and interaction of PI with environment (15% and 22%, respectively). The points on Figure 2 that are the poorest fit with the regression line contribute most to this interaction.

Differences in results are relatively greater for certain PIs when grown like a potato crop (tubers from tuberlings and grown in the field). The extra effort of producing tuberlings from seedlings and growing them in a Hawaii field would be worthwhile if it allowed identification of some PIs whose tubers exhibit much higher antioxidant content than when grown in artificial conditions, i.e. from greenhouse-grown seedlings. Such PIs would be of particular interest if they had other advantages for breeding.

An inspection of the results in Table 1 and Figure 2 reveals that the above scenario may be the case. Of most interest are PIs whose tubers are very high in AOA as field-grown tuberlings. This is true of both *S. pinnatisectum* PIs, which were very high in all treatments. But some PIs, like *S. microdontum* 473166, did not have consistent results under all conditions. For example, AOA for this PI in HFT (762 µg TE/gfw) was four times more than its value in WGS (179 µg TE/gfw). Likewise, *S. commersonii* 473411 exhibited an HFT value (792 µg TE/gfw) that was more than twice that of WGS (296 µg TE/gfw) for AOA. Tubers from HFT for *S. canasense* 310956 and *S. stenotomum* 29110 had approximately twice the AOA as WGS tubers. These species are much easier to use in breeding and less weedy than *S. pinnatisectum* and *S. jamesii*. If testing in the field is not done, the most useful germplasm could be missed.

Implications for Screening and Strategies

Botanical seed populations in the genebank may be segregating and thus show substantial phenotypic variation. If variation is high, the bulk sample value (i.e. the mean) will underestimate the potential of the population in terms of the highest individual expression levels for antioxidants in that population. However, any bulk with a high mean must either have uniformly high individuals or, if variable, have predominantly very high individuals. Similarly, any bulk with a low mean must have uniformly low individuals or predominantly very low individuals. Thus the strategy employed was to reduce the scope of screening to a practical size by bulking individuals within a population first to see if high bulk means in populations amenable to cultivar breeding were found. Since high population means were found in *S. canasense, commersonii, microdontum* and *stenotomum* (all easily hybridized with tuberosum), focusing on these particular populations for fine (genotype level) screening would be a reasonable next step toward identifying optimal high antioxidant breeding parents from exotic germplasm. Such work needs to be conducted in field conditions as was done in Hawaii. It would also be intriguing to pursue basic research on the physiological-genetic basis for why some germplasm does not exhibit its high antioxidant potential when grown under greenhouse conditions.

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