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A Possible Heterotic Threshold in the Potato and its Implications for Breeding

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Summary. Complex hybrids containing genomes from three different *Solanum tuberosum* Groups were synthesized (3-way hybrids), utilizing 2n gametes in 4x–2x crosses. Ten such families were compared to nine analogous two-Group (2-way) hybrid families and nine (1-way) families representing conventional Gp. Tuberosum breeding materials. The three types of crosses, representing three descending levels of heterozygosity, were placed in four field trials.

The 3-way hybrids were never significantly superior to the 2-way hybrids for vigor, yield, or tuber type. When yields were adjusted for maturity differences, the 3-way hybrids tended to be inferior to the 2-way hybrids for yield. This suggests that there may be a heterotic threshold in the cultivated potato, beyond which point more heterozygosity does not result in greater vigor or more yield.

While the 2-way and 3-way hybrids did not significantly differ from each other, they both dramatically surpassed the conventional 1-way crosses for vigor and yield (42%). The evidence of a possible heterotic threshold indicates that more sophisticated methods such as cell fusion and bilateral sexual polyploidization may not be necessary to exploit the full potential of the hybrid approach in the potato. A simple and direct "2-way" hybridization approach may be optimal, or at least would seem comparable with other hybrid approaches, and is a technology ready for immediate and widespread implementation.

Key words: *Solanum* – Potato – Heterosis – Hybrid vigor – 2n gametes

Introduction

Maintaining a high level of heterozygosity is crucial in the potato, as is demonstrated by the severe inbreeding depression resulting from selfing or haploid extraction in *Solanum tuberosum* (Krantz and Hutchins 1929; De Jong and Rowe 1971; Mendiburu et al. 1974). Conversely, the value of increasing heterozygosity in the potato is demonstrated by marked hybrid vigor in intertaxonomic Group hybrids (Glendinning 1969; Hanneman and Peloquin 1969; Mendiburu and Peloquin 1971; Tarn and Tai 1973; Cubillos and Plaisted 1976; De Jong and Tai 1977). In addition to the potato's severe inbreeding depression and its potential hybrid vigor, this crop's autopolyploid nature further demonstrates the significance of high levels of heterozygosity in the potato. For example, tetraploid cultivars have greater yield potential than diploid cultivars (Mendoza and Haynes 1976; Mendiburu and Peloquin 1977). This can only be attributed to the greater number of alleles per locus possible in the polyploid form, since colchicine doubling experiments have revealed no advantage in higher ploidy per se (Rowe 1967). Bingham (1980) has reviewed the research indicating that multi-allelic loci are crucial to the full expression of heterosis in polysomic polyploids.

The recognition that increasing heterozygosity and more multi-allelic loci can mean more yield stimulated the development of new potato breeding schemes aimed at maximizing heterozygosity. Chase (1963) and Mendiburu et al. (1974) have proposed "analytical" breeding schemes directed at maximal heterosis. Both proposals involve using four divergent diploid parents (including haploids from the two tetraploid taxonomic subdivisions of *S. tuberosum*, Gp. Andigena and Gp. Tuberosum). These are crossed to produce two different diploid hybrids which are, in turn, combined and polyploidized to create a tetraploid with genomes from the four different parents, thereby maximizing the number of tetra-allelic loci. Chase (1963) proposed selection and hybridization at the diploid level followed by polyploidization using colchicine-doubling and a final hybridization. Mendiburu et al. (1974) proposed a similar scheme, but they recognized that the amount of heterozygosity in the final hybrid was dependent upon the mode of polyploidization. They argued that colchicine doubling followed by hybridization only transferred 67% of the potential heterozygosity because of the normal tetrasomic gametic segregation of 1:4:1 (% heterozygous). They concluded that a superior mode of polyploidization would be to hybridize directly, using 2n gametes formed by first division restitution (FDR) in meiosis. In the potato, FDR results in roughly 83% of the potential heterozygosity being transmitted to the hybrid, making FDR clearly superior to colchicine doubling. Wenzel et al. (1979) pointed out the obvious extension of this approach by proposing the use of cell fusion as a method of transferring 100% of the diploid's heterozygosity to the tetraploid hybrid.

The breeding strategy of choice may not necessarily create the maximal level of heterozygosity, but rather the optimal level of heterozygosity. The heterosis reported thus far represents only the simplest of inter-Group hybrids, with greater levels of heterozygosity being untested, and further yield gains only hypothetical. It is not yet known whether the continued increase in heterozygosity within increasingly complex hybrids (having additional doses of more remote germplasm) will result in: a) further large gains in yield, b) rapidly diminishing gains in yield, or c) an eventual reduction in marketable yield due to negative interactions. Will there be a heterotic threshold? It is important to answer this question so that the full potential of the hybrid approach can be estimated, allowing breeding plans to be solidified and initiated. For these reasons, the following study was undertaken.

Materials and Methods

In order to determine the extent to which more heterozygosity results in more yield, 3-way hybrids were created which should be more heterozygous than previous potato hybrids. These 3-way hybrids combine the genomes of three taxonomic groups (Gp. Tuberosum, Gp. Andigena, and Gp. Phureja): the high-yielding capacity of Andigena-Tuberosum hybrids (Tarn and Tai 1973; Glendinning 1969; Cubillos and Plaisted 1976) is combined with the high-yielding capacity of Phureja-Tuberosum hybrids (Hanneman and Peloquin 1969; Mendiburu and Peloquin 1971; Mok and Peloquin 1975; De Jong and Tai 1977). These 3-way hybrids were contrasted with 2-way hybrids (Phureja-Tuberosum hybrids) and 1-way crosses (Tuberosum crosses), such that there were three levels of heterozygosity in the comparison. Nine 1-way families, nine 2-way families, and ten 3-way families were used in this study and are listed in Table 1.

The 1-way crosses, which involve the single taxonomic Group, Tuberosum, represent material which has traditionally been used in conventional breeding programs. The seed for

Table 1. Cross type, genomic designation, parentage, and source for 28 families used in the comparison of 1-way, 2-way, and 3-way crosses

Cross type	Family number	Genomic designation	Parentage	Source
1-way	1	TTTT	Bellisle × Wischip	Peloquin
1-way	2	TTTT	Merrimack × Platte	Peloquin
1-way	3	TTTT	Saco × Wischip	Peloquin
1-way	4	TTTT	Superior × New Haig	Peloquin
1-way	5	TTTT	W 623 × Kennebec	Peloquin
1-way	6	TTTT	W 718 × Katahdin	Peloquin
1-way	7	TTTT	W 718 × Kennebec	Peloquin
1-way	8	TTTT	W 726 × Lenape	Peloquin
1-way	9	TTTT	Wis. AG 231 × W 639	Peloquin
2-way	1	TTTP	Kennebec × US-W 5295.7	Peloquin
2-way	2	TTTP	N. D. 8891-3 US-W 5295.7	Peloquin
2-way	3	TTTP	W 623 × US-W 5295.7	Peloquin
2-way	4	TTTP	W 639 × US-W 5295.7	Peloquin
2-way	5	TTTP	W 707 × US-W 5295.7	Peloquin
2-way	6	TTTP	Wis. AG 231 × US-W 5295.7	Peloquin
2-way	7	TTTP	La-01-70 × US-W 5337.3	Peloquin
2-way	8	TTTP	WHS 17 × US-W 5337.3	Peloquin
2-way	9	TTTP	Wis. AG 231 × US-W 5337.3	Peloquin
				female from:
3-way	1	TATP	AT-9 × US-W 5295.7	Tarn
3-way	2	TATP	AT-14 × US-W 5295.7	Tarn
3-way	3	TATP	TA-17 × US-W 5295.7	Tarn
3-way	4	TATP	TA-25 × US-W 5295.7	Tarn
3-way	5	TATP	M-11-41 × US-W 5295.7	Plaisted
3-way	6	TATP	S 324.4 × US-W 5295.7	Plaisted
3-way	7	TATP	S 364-2 × US-W 5295.7	Plaisted
3-way	8	TATP	S 365-21 × US-W 5295.7	Plaisted
3-way	9	TATP	S 369-3 × US-W 5295.7	Plaisted
3-way	10	TATP	S 377-48 × US-W 5295.7	Plaisted

these nine families was supplied by Dr. S. J. Peloquin, Department of Horticulture, University of Wisconsin, Madison, Wisconsin, with the families being selected on the basis of having desirable, high-yielding parents.

The 2-way hybrids involve two taxonomic groups, Phureja and Tuberosum. Tuberosum haploids were crossed with diploid Phureja, and the resulting diploid hybrids were crossed to tetraploid Tuberosum via 2n gametes formed by FDR. The resulting tetraploid hybrids were three-fourths Tuberosum and one-fourth Phureja. Nine high-yielding Tuberosum clones were crossed with either of two elite haploid-Phureja hybrids which produce 2n pollen. The seed for these nine families was also supplied by Dr. S. J. Peloquin.

The 3-way hybrids involve three taxonomic groups, Andigena, Phureja, and Tuberosum. One of the same Tuberosum haploid-Phureja hybrids used in making the 2-way hybrids was used to make the 3-way hybrids, utilizing the same 2n pollen. However, instead of using Tuberosum cultivars as a female as in the 2-way hybrid, elite Andigena-Tuberosum hybrids were used. The resulting hybrids were one-half Tuberosum, one-quarter Andigena, and one-quarter Phureja; these differed from the 2-way hybrids only by the substitution of an Andigena genome for a Tuberosum genome. These crosses were made with the Tuberosum haploid-Phureja hybrid (US-W 5295.7) supplied by the Inter-Regional Potato Introduction Project at Sturgeon Bay, Wisconsin. The Andigena-Tuberosum hybrids were supplied by Dr. R. L. Plaisted, Department of Plant Breeding and Biometry, Cornell University, Ithaca, New York, and Dr. T. R. Tarn, Agriculture Canada, Research Station, Fredericton, New Brunswick, Canada. The Andigena in these hybrids had in both cases been selected for adaptation to the northern latitudes. These 3-way hybrids, along with the 1-way crosses and the 2-way hybrids, were put into the four different yield trials as described below:

1) 1978 Trial: Families from seedlings, Hancock: The 28 families representing the three types of crosses were grown as seedlings and were transplanted into the field at the University of Wisconsin Experiment Station at Hancock, which is located in the principal potato production region of the state. A randomized complete block design was used, with each of the 28 families replicated in four large blocks having nine seedlings per family per block, with a spacing of 30 cm between plants and 91 cm between rows. Within the larger blocks families were organized into nine sub-blocks, each containing a 1-way, 2-way, and 3-way family, with the tenth 3-way family at the end of each block. Tubers were harvested 103 days after transplanting to the field. At the end of the season, plants were harvested and weighed on a single hill basis to allow calculation of within-family variance. Visual scores were made at mid-season for each family's general vine vigor, at the end of the season for general vine maturity, and after harvest for general tuber type.

2) 1979 Trial: Selected clones grown from tubers, Hancock: The highest yielding four hills in each family of the first block of the 1978 seedling trial were selected and planted in 1979 at the Hancock location. Each selected clone was represented by a six hill plot, with 46 cm spacing between plants and 91 cm between rows. There were four such plots per family, with the same number of families per cross type as before, with one exception – a 1-way family, W726 × Lenape, was lost and could not be included in this trial. Therefore, there were 32 replicates (genotypes) for the 1-way cross type; 36 replicates for the 2-way cross type, and 40 replicates for the 3-way cross type. Each family of each cross type was included in four blocks of 108 plots each. Tubers were harvested 126 days after planting. Each plot was scored visually – twice for vine vigor, three times for vine maturity, and once for tuber type.

3) 1979 Trial: Selected clones grown from tubers, Sturgeon Bay: This trial was essentially the same as the previous Hancock trial, only at a different location. The highest yielding five hills in each family of the second block of the 1978 seedling trial were selected and planted in 1979 at the University of Wisconsin Peninsular Branch Experimental Station at Sturgeon Bay, Wisconsin. This location has a cooler climate than Hancock with a heavy and rocky soil. Each clone was represented by six hills with 81 cm between plants and 122 cm between rows. With five different clones per family, there were 45 replicates for the 1-way and 2-way cross types and 50 replicates for the 3-way cross type. Each family of each cross type was included in five blocks of 140 plots each. Tubers were harvested 135 days after planting. Vine vigor was scored once early in the season; vine maturity was scored three times late in the season, and tuber type was scored after harvest.

4) 1979 Trial: Families from unselected greenhouse-grown Tubers, Hancock: In 1978 at mid-season, a cutting was taken from each plant in the first block of the seedling trial and grown in the greenhouse to ensure virus-free plant material for the following year. The greenhouse-grown tubers were harvested in the fall. These tubers were planted in 1979, exactly replicating the first block of the seedling trial, such that each cross type was replicated over the nine families, which were blocked by cross type (9 blocks). This trial differed from the first in that it was grown from tubers rather than seedlings and the spacing between plants was 61 cm rather than 30 cm, with 91 cm between rows as before. Tubers were harvested 126 days after planting.

Statistical analysis was performed using the Minitab computer program, with a single factor analysis of variance. Although all experiments were carefully blocked, block effects were generally very small, and for convenience, all experiments were analyzed as completely randomized designs. Bartlett's test for homogeneity indicated significant differences between treatment variances. This was corrected using a log transformation of the data. For clarity of presentation, however, the data is reported in its original non-transformed form, but all statistical differences and correlation coefficients were confirmed using transformed data.

Results

1) 1978 Seedling Trial: The results of the 1978 seedling trial are summarized for the three types of crosses in Table 2. Included in Table 2 are the summary statistics for the six 2-way families having US-W 5295.7 as a male parent, as well as the summary statistics for all nine 2-way families. Because these six 2-way families have the same male parent as the 3-way families, their summary statistics may be used to make a fairer 2-way vs. 3-way comparison.

The 2-way hybrids, taken as a whole, yielded 33% more than the traditional 1-way crosses, whereas the 3-way hybrids yielded 40% more than the traditional crosses and 6% more than the 2-way hybrids. Superiority of the hybrids over the 1-way crosses was highly significant, while the difference between the 2-way and 3-way hybrids was not significant. When just the 2-way

Table 2. Summary statistics for 1-way, 2-way, and 3-way families; for mean yield, mean within-family coefficient of variability for yield, mean vine vigor, mean vine maturity, and mean tuber type scores – in four yield trials

Field Trial	Cross type	Mean yield (grams/hill)	Mean C.V. within-families	Mean general vine vigor[a]	Mean general vine maturity[b]	Mean general tuber type[c]
1. Seedling trial, 1978, Hancock	1-way	670 a[e]	58.8% a	2.48 a	1.77 a	3.98 a
	2-way	890 b	47.7% b	4.02 b	2.13 a	3.09 b
	2-way[d]	940 b	46.8% b	3.92 b	1.95 a	3.27 b
	3-way	940 b	49.9% b	3.46 b	1.95 a	3.20 b
	lsd 0.05	145	7.68	0.95	n.s	0.582
2. Selected clones, 1979, Hancock	1-way	2,030 a	23.2% a	2.63 a	2.85 a	3.48 a
	2-way	2,880 b	19.0% a	3.14 b	3.15 a	2.88 ab
	2-way[d]	2,780 b	18.2% a	3.00 ab	2.82 a	2.86 ab
	3-way	2,910 b	19.7% a	3.17 b	3.43 a	2.20 b
	lsd 0.05	486	n.s.	0.450	n.s.	0.648
3. Selected clones, 1979, Sturgeon Bay	1-way	2,590 a	32.9% a	2.36 a	2.58 a	3.49 a
	2-way	3,300 b	31.0% a	2.89 b	2.90 a	2.20 b
	2-way[d]	3,490 b	27.5% a	2.83 b	2.67 a	2.30 b
	3-way	3,390 b	27.1% a	2.82 b	3.00 a	2.10 b
	lsd 0.05	406	n.s.	0.399	n.s.	0.444
4. Unselected tubers, 1979, Hancock	1-way	2,020 a	46.7% a			
	2-way	3,140 b	47.3% a			
	2-way[d]	3,200 b	45.4% a			
	3-way	3,250 b	37.9% a			
	lsd 0.05	548	n.s.			

[a] Vine vigor was scored as follows: 1 = very weak, 5 = very strong
[b] Vine maturity was scored as follows: 1 = very early, 5 = very late
[c] Tuber type was scored as follows: 1 = very poor, 5 = very good
[d] Only the six 2-way families with US-W 5295.7 as a male parent
[e] Means within the same column, within each field trial, followed by the same letter are not signifiantly different at the 5% level using Fisher's LSD

families having US-W 5295.7 as a parent were considered, the 2-way and 3-way mean yields were identical, both being 40% superior to the 1-way yields.

Both types of hybrids had significantly greater vine vigor than the 1-way crosses, while not differing significantly from each other. Likewise, for tuber type scores, both the 2-way and 3-way hybrids were significantly inferior to the 1-way crosses, while not differing significantly between each other. There were no significant differences for vine maturity between the three cross types.

Yield variance tended to increase with increasing yield, such that the hybrids, with their higher yields, had the greatest total variance and the greatest between-family variance. However, the mean within-family variance of the hybrids was not significantly greater than that of the 1-way crosses. In terms of the coefficient of variability (Table 2), the hybrids had significantly less within-family variability than the 1-way crosses. Since the coefficient of variability is used as a relative measure of variation, it may be concluded that the hybrid families were relatively more uniform than the conventional families.

2) 1979 Trials: Selected hills grown from tubers. Hancock: When selected clones were grown from tubers at Hancock, the 2-way and 3-way hybrids again significantly out-yielded the conventional 1-way crosses, this time by 42% and 43%, respectively. The 3-way hybrids did not yield significantly more than the 2-way hybrids.

The 3-way hybrids and the 2-way hybrids (taken as a whole) both had significantly greater vine vigor than the 1-way crosses. The 3-way hybrids had significantly inferior tuber type compared to the 1-way and 2-way crosses, while the 2-way hybrids did not differ significantly from the 1-way crosses for this trait. There were no significant maturity differences among the three cross types.

Total yield variance and between-family variance were both considerably greater in the hybrids than in the 1-way crosses, while the mean within-family variance was not, as was also observed in the seedling trial. Although in the previous seedling trial the within-family coefficient of variability was significantly less in the hybrids than in the 1-way crosses, this difference was not large enough in this trial to be significant, although the data suggest the same trend.

3) 1979 Trial: Selected hills grown from tubers, Sturgeon Bay: When selected clones were grown from tubers at Sturgeon Bay, the error variance was the highest and the relative advantage of the hybrids over the conventional crosses was the lowest of all four trials. This response was due to a less uniform trial environment with greater soil heterogeneity than at Hancock, and differential damage from the herbicide metrabuzin. Nevertheless, yields per hill were higher at this location than at Hancock, which has been attributed in part to wider spacing between plants.

As in the previous trials, the 2-way and 3-way hybrids significantly out-yielded the conventional 1-way crosses, but this time only by 27% and 31%, respectively. The 3-way hybrids did not significantly out-yield the 2-way hybrids, and when only families with US-W 5295.7 parentage were considered, the 2-way hybrids had a slight but insignificant yield advantage over the 3-way hybrids.

The 2-way and 3-way hybrids had significantly greater vine vigor and inferior tuber type compared to the 1-way crosses, as generally observed in the previous two trials. As before, the 2-way and 3-way hybrids did not significantly differ between themselves for either trait.

The total yield variance and between-family variance tended to be greatest in the hybrids in association with their higher yields. The hybrids did not have significantly higher within-family variance. While the within-family coefficients of variability were not significantly less in the hybrids than in the conventional crosses, the data indicate this trend as in the other trials.

4) 1979 Trial: Families from greenhouse tubers: When an unselected replicate of the seedling trial was grown from tubers at Hancock, mean yields per hill were roughly 300% higher than the seedling trial, presumably due to propagation from tubers rather than seedling transplants and the wider spacing between plants. As in the first year, the 2-way and 3-way hybrids significantly out-yielded the 1-way crosses, this time by 55% and 61%, respectively, the largest difference in the study. The differences between the 2-way and 3-way hybrids were not significant, and when only families with US-W 5295.7 parentage were considered, the 2-way and 3-way mean yields were essentially identical, as observed before.

Yield was the only character measured in this trial. The differences in between-family variance for yield were not as clear as in the seedling trial, and the three cross types did not differ greatly for either the mean within-family variance or the mean within-family coefficient of variability; however, there was less replication in this trial relative to these traits.

Summary of the Results from the Four Trials

When the four different yield trials are taken together, a consistent pattern is clearly visible. In each trial the hybrids clearly out-yielded the 1-way crosses and when averaged over the four trials, the hybrids had yields 42% greater than the conventional crosses. The 3-way hybrids never yielded significantly more than the 2-way hybrids, with the average yield difference over all experiments being only 3%. When considering just those families with US-W 5295.7 parentage, the 2-way and 3-way average yields over all experiments were essentially identical, with only a 20 gram difference between them. The distributions of family mean yields, averaged over the three Hancock trials, are illustrated in Fig. 1.

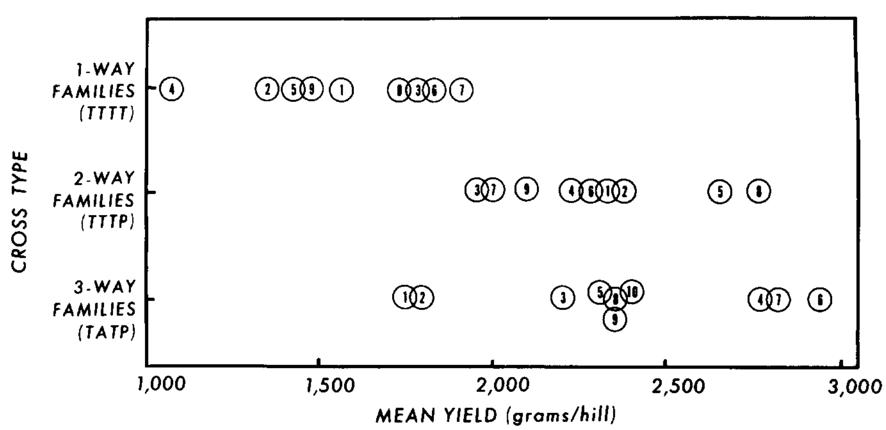

Fig. 1. Distributions of family mean yields for 1-way, 2-way, and 3-way crosses when averaged over the three yield trials at Hancock, Wisconsin. Circled numbers designate the numbered families listed in Table 1. 2-way families 7, 8, and 9 had US-W 5337.3 as the male parent; 3-way families 1, 2, 5, 6, and 7 were in Andigena cytoplasm

The distributions for the mean yields of the 1-way and 2-way families do not over-lap, with the lowest 2-way family out-yielding the highest 1-way family.

Unlike vine maturity scores, the mid-season vine vigor scores always revealed significant differences. While the 2-way and 3-way hybrids never significantly differed for vine vigor, they were both consistently superior to the 1-way crosses for this trait, and were consistently inferior for tuber type (with a single 2-way exception at Hancock).

The hybrids were characterized by higher total and between-family variance. At the same time, the mean within-family variance was never significantly higher in the hybrids than in the 1-way crosses, and the mean within-family coefficient of variability of the hybrids tended to be less than that of the conventional families, with this difference being significant in the seedling trial.

Mean yields Adjusted for Maturity Differences

Although the Andigena used in the 3-way hybrids had been selected for adaptation to the northern latitudes, it could be argued that this germplasm may have detracted from the local adaptation of the 3-way hybrids by causing excessively late maturity. The analyses of variance did not reveal any significant differences for vine maturity as scored visually, but the 3-way hybrids did have slightly later vine maturity scores than the 2-way hybrids. Conversely, the higher yields of the hybrids might be attributed to their later maturity since they did have somewhat later maturity scores than the conventional crosses. To eliminate this ambiguity, correlation coefficients were calculated between yield and maturity scores, and adjusted mean yields were calculated by regression, correcting for maturity differences.

Correlation coefficients and adjusted mean yields are summarized in Table 3. Correlation coefficients were small, indicating that maturity differences had a limited effect on yields. More importantly, yield/maturity correlations were always positive, indicating that later maturity enhanced yields, and that the 3-way hybrids were in no way penalized for their slightly later maturity.

The data from the 1979 trial from unselected tubers, while displaying the greatest heterotic yield advantage for the hybrids over the traditional crosses, could not be adjusted for maturity since the maturity data were not available. Excluding this trial, the average unadjusted yield advantage of the hybrids over the 1-way crosses was 35%, and after yields were adjusted for maturity, this difference was reduced to 24%.

The superiority of the 2-way hybrids over the 1-way crosses was little affecting by adjusting yields for maturity, with its superiority being 34% before and 29% after the adjustment. The average yield difference between the 1-way and 2-way crosses was 594 grams before and 540 grams after the adjustment, indicating that 9% of the original superiority of the 2-way hybrids was due to later maturity and 91% was due to true (or at least maturity-independent) heterosis. After yields were adjusted, the 2-way hybrids remained significantly superior to the 1-way crosses in all trials.

The 3-way hybrid yields were greatly reduced by adjusting for maturity. While the superiority of the 3-way hybrids over the 1-way crosses was 37% before adjustment, it was only 20% after adjustment. Using the same type of calculations as with the 2-way hybrids, it can be seen that 43% of the 3-way superiority was due to its later maturity, and only 57% of this superiority was due to true or maturity-independent heterosis. After yields were adjusted, the 3-way hybrids did not yield significantly more than the 1-way crosses in the Stur-

Table 3. Yield-maturity correlations, and mean yields of 1-way, 2-way, and 3-way crosses after adjustment for maturity differences for three trials

	Seedling trial Hancock, 1978 (grams/hill)	Selected clones Hancock, 1979 (grams/hill)	Selected clones Sturgeon Bay, 1979 (grams/hill)
yield-maturity correlation, r	0.33 n.s.	0.22*	0.33**
1-way adjusted mean yield	714 a†	2,108 a	2,790 a
2-way adjusted mean yield	923 b	2,979 c	3,330 b
3-way adjusted mean yield	868 b	2,699 b[1]	3,150 ab
lsd 0.05	135	333	394

* Significant at the 5% level
** Significant at the 1% level
† Means within the same column, followed by the same letter are not significantly different at the 5% level using Fisher's LSD, except as noted
[1] Mean separation based on lsd 0.10

geon Bay trial and yielded significantly less (at the 10% level) than the 2-way hybrids in the 1979 selected hills trials at Hancock.

Discussion

Two important points are made clear by the results of these four trials. First, both types of hybrids have markedly higher yield potential than the conventional crosses, and secondly, the 3-way hybrids were in no way superior to the 2-way hybrids. These two facts seem to indicate both the potential and the possible limitations of the concept of increasing heterozygosity.

2-Way vs. 3-Way Hybrids

In this study, the 3-way hybrids were synthesized specifically to determine if the high yields of the 2-way hybrids used were from 4x–2x crosses, with the same type of Phureja-haploid hybrid used as the diploid male, producing FDR 2n pollen. The only difference in the hybrids were used from 4x – 2x crosses, with the same type of Phurejahaploid hybrid used as the diploid male, producing FDR 2n pollen. The only difference in the 3-way hybrids was that rather than having Tuberosum as the female tetraploid, selected Andigena-Tuberosum hybrids were used such that the 3-way progeny had an Andigena genome substituted for one of the Tuberosum genomes in the 2-way hybrids.

The "relative genetic distance" (percent proteins not in common) between the four taxonomic Groups of *S. tuberosum* can be estimated using electrophoretic data generated by Rickeman and Desborough (1978). This data, in conjunction with prevailing taxonomic views, suggests that Phureja and Stenotomum are closely enough related so that from the point of view of heterozygosity, one could consider them to be the same Group. A 3-way hybrid with genomes from all three major *S. tuberosum* Groups (Tuberosum, Andigena, and Phureja/Stenotomum) would seem to incorporate what is approaching the maximum amount of heterozygosity available, short of using wild species as parents.

Since the heterozygosity of a gamete in a polysomic polyploid is a function of a) the parental heterozygosity and b) the transmission rate of that heterozygosity through the meiotic process, one can compare the relative heterozygosity of the gametes used in this study. The heterozygosity of the Phureja-haploid hybrids is based upon a genetic distance of '57' (Rickeman and Desborough 1978), with a rate of transmission through the FDR 2n gametes of 83% (Mendiburu et al. 1974). Therefore, one can assign these gametes a relative heterozygosity value of 47.3. The heterozygosity of the gametes from the Andigena-Tuberosum hybrids is based upon a genetic distance (percent of proteins different) of '43' (Rickeman and Desborough 1978), with a rate of meiotic transmission of heterozygosity of 67%. This rate is based upon common tetrasomic segregation wherein the tetraploid hybrid, TTAA, segregates in meiosis in a 1:4:1 ratio for gametes TT, TA, AA. In actuality, this four of six, or 67% transmission rate is an underestimate of the heterozygosity in the Andigena-Tuberosum gametes since a certain fraction of the AA and TT gametes should also be heterozygous. Ignoring this extra heterozygosity, one can still assign the Andigena-Tuberosum gametes a minimum relative heterozygosity value of 28.8. Such Andigena Tuberosum gametes, therefore, have at least 61% as much heterozygosity as the Phureja-haploid 2n gametes. Since the Phureja-haploid 2n gametes had a pronounced effect on heterosis, the Andigena-Tuberosum gametes might be expected to make a lesser, but still significant, contribution to heterosis and yield.

Using the conventional Tuberosum × Tuberosum crosses as a standard, the 2-way hybrids contributed through the male gamete an additional dose of heterozygosity with a relative value of 47.3, and tuber yield was consequently increased 42%. The 3-way hybrids provided through the female gamete an additional contribution in heterozygosity with a relative value of 28.8, but the yield only increased an additional 3%, which was not significant. This suggests a possible heterotic threshold, at which point further increases in heterozygosity will not result in greater heterosis or yield. The theoretical implications of this conclusion must be tempered by the fact that the specific combining ability of Phureja and Tuberosum seems to be higher than that of Andigena and Tuberosum. Therefore, Andigena would not be expected to make as large a heterotic contribution as Phureja in these crosses. However, this cannot explain the absence of heterosis in the 3-way crosses over the 2-way crosses.

While consideration of the specific combining ability of these taxonomic groups may affect our theoretical interpretation of a heterotic threshold, the applied implications of these results are unambiguous. Since there are no other cultivated Groups to use as parents in potato breeding, either the 3-way specific combining ability tested in these trials is better than the 2-way specific combining ability or it is not. Only by using wild species as parents could heterozygosity be greatly increased above the 3-way crosses, and thus far, there is no evidence that wild species have high combining ability for yield in direct crosses with *S. tuberosum*.

It might be argued that the slightly later maturity of the 3-way hybrids prevented the expression of their full yield potential, thereby favoring the 2-way and 1-way crosses. This argument is not consistent with the vine vigor scores, which should not have been affected by maturity differences and which also failed to reveal any heterosis of the 3-way over the 2-way hybrids. Furthermore, this maturity argument can easily be proven false, since the correlation between later maturity and higher yields was always positive, with later types yielding more. In fact, when yields were adjusted for maturity differences, the 3-way yields were always less than the 2-way yields.

It may also be argued that this study could have been confounded by cytoplasmic influences, since five of the ten 3-way families were in Andigena cytoplasm. There is evidence that Andigena cytoplasm may contribute to a decrease in yield, thus, the 3-way crosses may have been unduly penalized. This possibility was examined and it was found that the five 3-way families in Tuberosum cytoplasm did yield slightly more than the five 3-way families in Andigena cytoplasm. However, this difference was not statistically significant, and when the data was reanalyzed using only families in Tuberosum cytoplasm, cross type mean yields were essentially the same and statistical differences were unchanged (Fig. 1). It is important to note that the largest reciprocal differences reported in the literature have been when unselected Andigena has been used as a parent, whereas when selected day-neutral Andigena has been used (as in the present study), reciprocal differences have been small or absent (Sanford and Hanneman 1979, 1981). Furthermore, the crosses in the current study are equivalent to backcrosses away from Andigena, and in such instances, reciprocal differences have been found to be greatly reduced or absent (Sanford and Hanneman 1979, 1981).

The Value of the Hybrid Approach

Although there were few significant differences between the 2-way and 3-way hybrids, there was no difficulty in detecting significant differences between the hybrids and the conventional crosses. The hybrid families were consistently superior to the conventional crosses in mean yield, adjusted mean yield (with a single exception) and vine vigor. Figure 1 vividly demonstrates the yield superiority of the hybrids.

The 42% yield superiority of the hybrids, averaged over the four trials, is consistent with the high degree of mean heterosis previously reported in $4x-2x$ crosses (Hanneman and Peloquin 1969; Mendiburu and Peloquin 1971; De Jong and Tai 1977; Mok and Peloquin 1975; Tai 1979). These previous trials, in conjunction with the current study, leave no doubt that the yield potential of the potato can be substantially increased using the $4x-2x$ approach.

Not only do the hybrid families have superior mean yields, but they also are characterized by greater total yield variance and between-family variance. It should not be surprising that a crop that has been characterized as having a very narrow germplasm base (Simmonds 1969; Mendoza and Haynes 1974) should benefit from the infusion of new genes. Selection within the hybrids should be more productive because of the wider genetic range and also because the greater genetic variance should increase heritabilities.

These hybrids may also find novel applications in production. The greater vine vigor of the hybrids may not just result in stronger plants, but may have direct economic value, for example, as silage (Parfitt and Peloquin 1977; Nicholson et al. 1978). As observed in earlier trials, hybrid family means can be superior to highly selected cultivars. This, in conjunction with the greater relative uniformity within hybrid families (as suggested by the significantly reduced within-family coefficients of variability in the seedling trial), may help to make potato production from botanical seed become a reality in tropical areas (Anon. 1978).

The high productivity of the hybrids may find immediate application in areas where rigid quality standards have not yet developed. However, by North American standards, tuber quality in the hybrids was clearly inferior to the 1-way crosses. Traits such as deep eyes, undersized tubers, and extra pigmentation must be eliminated before the high yields of the hybrids can be transformed into marketable yield. However, there are both diploid and tetraploid hybrids with good tuber quality, and there is good reason to believe that these traits will respond rapidly to selection. More Phureja-haploid hybrids with good tuber type need to be developed.

This study has revealed an apparent heterotic threshold, indicating that the simple and direct $4x-2x$, 2-way hybrid approach should have at least comparable biological potential with the more complex breeding schemes such as cell fusion, bilateral sexual polyploidization, and 3-way and 4-way hybrids. Furthermore, the 2-way approach, because it is simple and direct, has the greatest potential of being widely applied and should allow the maximum amount of genetic diversity to be synthesized and screened. Unlike more complex hybrid breeding schemes, the simple 2-way approach is a technology which is ready for immediate implementation.

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