Examination of Maximilian Sunflower (*Helianthus maximiliani* Schard.) Ideotypes with Restricted Branching

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Introduction:

Perennial grains have been suggested as a potential route for enhancing the sustainability of agricultural systems through the contribution of new crop ecosystems. Efforts are underway to develop new perennial crops forms. Breeding strategies for perennial grains and oilseeds follow two main paths: i) the perennialization of existing crops; and ii) the neo-domestication of wild perennial species.

Maximilian sunflower (*Helianthus maximiliani* Schrad.) is a herbaceous perennial wild species, a relative of domesticated sunflower (*Helianthus annuus* L.) and a candidate for neo-domestication. Maximilian sunflower is widely adapted across the central plains of North America and exhibits both abundant seed and rhizome production once established, making it a good perennial oilseed candidate. The biology and agronomic characterization of this species is currently limited and plant ideotypes for agricultural production are undefined.

Wild populations exhibit profuse branching, small capitula, and uneven maturity of capitula (Figure 1). A population developed by The Land Institute (TLI) exhibits a restricted branching architecture with larger capitula or a single central capitulum. Selection for restricted branching and subsequent increase in capitula size observed in TLI lines suggest a potential resource allocation trade-off between branch number, capitulum size and capitulum number.

The purpose of this research was to examine the relationship between branching characteristics and potential yield component trade-offs in a F_2 population developed from TLI and Manitoba wild type (MWT) crosses segregating for branch number.

Materials and methods:

To generate a F_2 population segregating for branching characteristics, reciprocal crosses were performed between a single unbranched TLI institute plant exhibiting a single large capitulum and a highly branched MWT plant with small capitula (Figure 2). As Maximilian sunflower is self-incompatible F_1 progeny were vegatatively cloned and crossed in a pairwise fashion to determine compatible F_1 individuals.

Two pairs of reciprocal F_1 crosses exhibiting adequate seed set were selected for F_2 phenotyping. Parental and F_1 vegetative clones along with F_2 plants derived from seed were grown under growth room conditions in 1 liter pots consisting of a 2:2:1 sand:soil:peat mixture following a 23°C 16hr light/18°C 8hr dark regime. To ensure good branching development, plants were fertilized at regular two week intervals using full strength 20:20:20 fertilizer and rotated throughout the growth room to reduce potential edge effects and maintain uniform plant spacing.

Plants were phenotyped when five capitula had reached anthesis, or if less than five capitula were produced, one week following anthesis of the last capitulum. A total of 250 plants were phenotyped for a series of traits including days to anthesis of the first through fifth capitula, total branches, average branch length in cm, total main stem nodes, average capitulum size (diameter in mm), stem diameter in mm and total capitulum count.

Plants were divided into branching classes based upon their branch number for LSmean comparisons. As classes 1-4 and 14-18 were found at low frequencies they were merged into a highly restricted (HR) and Highly branched (HB) classes respectively. Statistical analysis was conducted using SAS 9.3 (SAS, 2011) and JMP 11(SAS, 2013) LSmean for days to anthesis of the fifth capitulum, capitula count, stem diameter, capitula size, total nodes and branch length were calculated for each class using SAS PROC MIXED . The model consisted of Branch count = trait + random effects + error. Branch count was classified as a fixed effect while cytoplasm and cross served as random effects.

Data were log transformed when deemed appropriate and outliers by branching group were removed based on visual assessment. Mean, range, standard deviation and coefficient of variation values were calculated using SAS PROC MEANS. Correlations were calculated using SAS PROC CORR and principal component analysis was run using JMP 11. Analysis of parental and F_1 phenotypic values and heritability estimates using parent-offspring regression were not included directly in the analysis of the F_2 population due to the limited size of the parental and F_1 populations and potential differences in ontogeny between vegetative clones and seed derived populations.

Figure 1: MWT plants in early bloom at 1.8m spacing, two years following initial transplantation

Figure 2: Left: TLI line exhibiting increased capitulum size, Right: MWT individual exhibiting a small capitulum

Results:

Capitula count had the highest coefficient of variation (C.V.) amongst the examined traits followed by average branch length, while all other traits appeared to have a relatively small C.V. (Table 1). The unbranched characteristic of the TLI parent was not recovered in either the F_1 or F_2 generations (data not shown), although segregation for branching traits was apparent in the F_2 (1-18 branches).

Based upon LSmean analysis, branching class appears to influence days to anthesis, capitulum count, stem diameter and average branch length. Average capitulum size and total nodes do not appear affected by branching class.

Average capitulum size did not differ significantly across branching classes.
Plants in the restricted branching class (1-4 branches) had significantly fewer capitula than all other branching classes, there were no significant differences between all other branching classes (5-HB classes) for this trait.

Correlation analysis showed a significant correlation between several traits; branches showed a significant correlation with days to anthesis, capitula count, capitula size and average branch length (Table 2).

Principal component analysis revealed seven components explaining the cumulative variation in the dataset (Table 3). The first principal component, explaining 30.6% of the total variation, was dominated by anthesis followed by total capitula, branches and capitula size, respectively. The second principal component was dominated by stem diameter and accounted for 19.6% of the variation, while the third principal component, dominated by branch length explains 18.6% of the total variation. The first three principal components account for 68.8% of the total variation (Figure 3) while the 4th-7th principal components account for the remaining 31.2% of the variation in the dataset.

Table 1: Summary statistics for trait variables

Trait	Mean	Range	Std Dev	C.V.
Days to anthesis (days)	170.7	138	24.6	14.4
Capitula count	32.0	86	14.6	45.6
Stem diameter	9.4	14	1.3	14.8
Average capitula size (mm)	13.1	14	2.5	19.1
Total main stem nodes	49.6	67	10.4	21.0
Average branch length (cm)	30.7	54	9.7	31.7

Table 2: Correlation coefficients between select traits

Trait	Anthesis	Total Capitula	Capitula size	Branch length	Stem diameter	Branches
Total Capitula	-0.28*					
Capitula size	0.19*	-0.29*				
Branch length	NS	NS	0.27*			
Stem diameter	NS	NS	0.23*	0.13*		
Branches	-0.33*	0.52*	-0.18*	-0.13*	NS	
Nodes	0.68*	NS	0.23*	NS	NS	NS

*Denotes significance at D.f= 249 and alpha=0.05, NS denotes = non-significant

Table 3: Summary of trait eigenvectors and percent variation explained by principal components

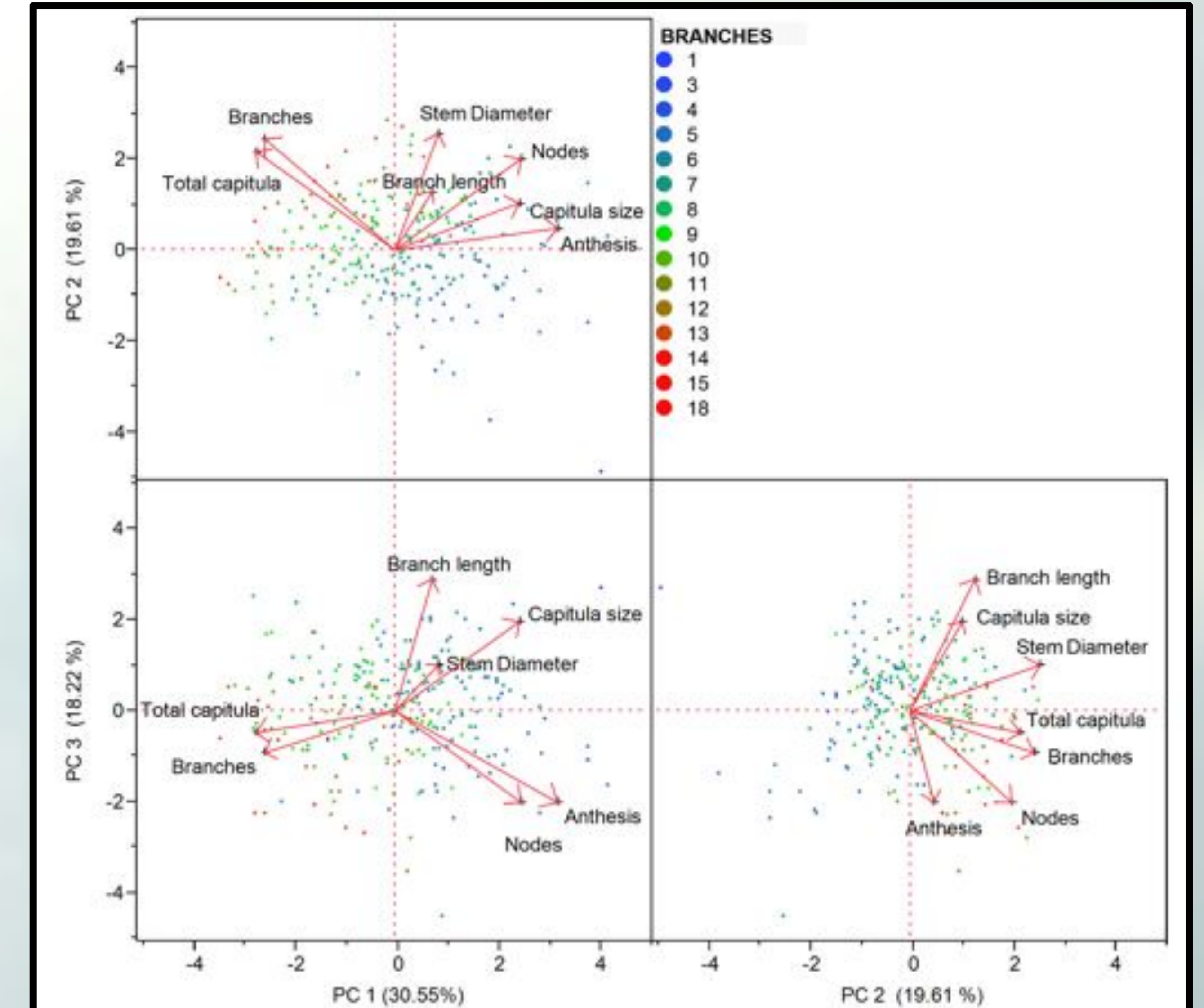|	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Total Capitula	-0.44	0.44	-0.1	0.33	-0.12	0.68	-0.01
Capitula size	0.39	0.2	0.41	-0.11	0.68	0.36	-0.12
Branch length	0.12	0.26	0.61	0.58	-0.27	-0.33	0.13
Branches	-0.41	0.49	-0.19	-0.12	0.37	-0.48	0.38
Nodes	0.4	0.4	-0.42	0.28	0.15	-0.18	-0.59
Stem Diameter	0.13	0.51	0.21	-0.65	-0.46	0.02	-0.14
Anthesis	0.52	0.09	-0.42	0.09	-0.25	0.13	0.66
Percent variation	30.55	19.61	18.22	12.59	9.68	5.64	3.67

Figure 3: Biplot representation for principal components 1-3 representing 68.8% of the total variation in the dataset. The coloured points represent branch count as a frame of reference.

Discussion:

Selection for reduced branching may limit total capitula count at less than five branches. While capitula count was limited in the restricted branching class, capitula size was not. Negative correlation between capitula size and capitula count may represent a partial trade-off between these traits. The C.V. of capitula count was higher than the for other traits and may present a greater degree of plasticity relative to traits such as capitula size. In field studies of Maximilian sunflower capitula count appears to be plastic in nature while average capitula size appears to be relatively fixed (data not shown). Selection for capitula size over capitula number may help maximize potential seed set as capitula size appeared to be less plastic than capitula number.

Decrease in capitula number in the restricted branching class was not compensated for by a significant increase in capitula size. Simultaneous selection for capitula size and capitula number may be possible in plants with greater than four branches as they did not appear to be restricted in these classes. As branches did not significantly impact capitula size this relationship suggested that branching and capitula size were not genetically linked.

The first principal component may represent the effect apical dominance and a limited level of recombination in a F_2 population. The TLI parent exhibited strong apical dominance, with a large central capitulum, restricted branching and later anthesis, while the MWT parent exhibited profuse branching, small capitula and early flowering.

The second principal component likely represented the effect of plant vigour as all traits except for anthesis and capitula size (reduced in the MWT parent) increased along the same axis. This component may also represent recombination between the MWT branching traits (branch count, branch length, capitula count) and TLI parent traits (stem diameter, nodes).

The third principal component may represent a relationship between plant size (nodes) and branch length, capitula size and stem diameter. This may represent recombinants for parental capitula size and anthesis values as these traits showed a weak correlation (r=0.19). The fourth principal component may represent recombinants for stem diameter and branch length traits. The fifth and sixth principal components showed potential positive relationships between capitula size and branches, and capitula size and capitula number suggesting recombination.

Conclusions:

- Selection for an ideotype of five branches may minimize branching without a significant loss to either total capitula count or capitula size.
- Favorable recombinants observed in the F_2 population suggests it may be possible to select for increased seed yield due to the combination of increased capitula size and increased capitula number from the TLI and MWT parents, respectively.
- Selection for increased total capitula count as well as capitula size may be possible, though further generations of recombination may be required to break potential genetic linkage.

Ongoing and future work:

- Examination of the role of population structure on trait linkages and QTL mapping of favorable traits in this population employing genotype-by-sequencing (GBS).
- Selection of F_2 plants for open pollination and F_3 population development.
- Further examination of yield component traits such as floret size, seed yield per capitulum and seed yield per plant from replicated field trials is needed.

References:
SAS Institute, Inc. "SAS® 9.3." (2011).
SAS Institute., Inc. *Using JMP 11*. SAS Institute, (2013).

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