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***Helianthus annuus* natural populations to increase the whole genetic diversity of domesticated sunflower: the concept of neodomestication**

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ABSTRACT

To broaden genetic basis of cultivated crop, seventy-seven *Helianthus annuus* natural ecotypes from North America were grown in Mauguio near Montpellier and crossed with three domesticated sunflower lines. The crossed F₁ progenies were conducted in maternal lineage to maintain 10 plants per lineage in isolation for pollination and the intermixing was continued till G₄ generation under low to high selection pressure for some domestication traits. The G₀ generation enabled to phenotype thirteen morphologic descriptors. Some traits: phenology, plant height, branching, oil content, seed size and yield were evaluated through the G₁, G₂, G₃ and G₄ cycles. The studies of the first generations enabled us to release some main trends which are promising for the future of sunflower breeding. Indeed, we showed that the neo-domesticated materials gather adaptive traits from the natural populations. The first immediate effect of the intraspecific crossing between wild *H. annuus* and cultivated lines (G₀ -> G₁) was to increase 1000 seeds weight (1000SW) by 3.6 (9.7 g to 35.2 g), oil content by 20% (26.8 % to 32.3 %), and to reduce apical, intermediate and basal branching, respectively, by 53%, 40% and 13%. In the last G₃ and G₄ cycles, strong selection pressures were applied on the combined (1000SW*oil content) trait. Oil content increased by 3% in G₃ and continued by 11% in G₄. In the same time 1000SW, after small decrease, exhibited strong seed size increase (13%) in G₄ cycle. The other visible effect was a clear evolution towards domestication traits, i.e., decrease in the different types of branching, reaching 76% non branchy plants in G₄. Qualitatively, the different types of branching declined in the same way in G₄ cycle: 14% plants were apical branched, 11% intermediate and 17% basal branched.

Key words: domestication – genetics resources – neodomestication– wild sunflower.

INTRODUCTION

Wild sunflowers spread naturally in North America where they thrive as populations covering most of the environments from sea to mountain. Heiser (1947) reported that wild sunflowers were found throughout most of North America on disturbed, mesic, heavy soils that are wet in the spring but dry out by midsummer. The *H. annuus* natural populations reveal a particular interest for adaptation to biotic and abiotic environments (drought, cold tolerance). Rust, Alternaria and downy mildew resistances are frequently observed in that wild compartment. In screening downy mildew tests performed at INRA we observe that most of the wild accessions (61/72) carried resistance factors to downy mildew (race 710).

Broadly, wild sunflowers did not exhibit meiotic abnormalities in crosses with cultivars like other annual *Helianthus* species that carry genomic rearrangements in comparison to sunflower. Consequently, one can expect efficient intermingling of the cultivated and wild genomes along recurrent crosses. Moreover, the wild sunflower is strictly self-incompatible, whereas crop lines are self-compatible, which also favours intermingling of genomes.

Sunflower is an oil crop and breeding has dealt mainly with increasing oil yield. Oil yield has been increased drastically in Russia (Vrânceanu, 2000) and oil sunflower spread worldwide. In Russia, oil improvement has been accompanied by broomrape resistance since broomrape was prevalent in this country (Vrânceanu, 2000). To become an industrial crop sunflower diversity has been reduced for crop adaptation to hybrid seed production through a CMS (Leclercq, 1971) that infers splitting lines in A (male sterile), B (maintainer) and R (restorer) classes, to diseases, mainly downy mildew (Leclercq, 1971) and after Phomopsis spread (Škorić, 1985). Recently, the introduction of Pervenets mutation in the classic sunflower to obtain seed oil with high oleic acid content has revealed the narrowness of genetic diversity in the crop (Tang et al., 2003).

Until now, the method used to enlarge the genetic diversity for agronomic traits in sunflower crop was to cross sunflower with a wild sunflower carrying the useful trait and to evaluate progenies for the

trait under breeding. Similarly, other *Helianthus* annual species have been used in this way to introgress useful traits in sunflower such as Rf genes: (Jan and Seiler, 2007); Phomopsis (Škorić, 1985; Griveau et al., 1992; Serieys et al., 1998), and fertility (Quillet et al., 1992). This method has, as a main advantage, the limitation of the material to be examined in the field, but it also has as a main defect that only one main trait can be considered in each cross. Moreover, introgression lines keep only a few of the foreign genomes, which are eliminated to restore male fertility required in agreement with cultivation. We therefore conceived a method to enlarge genetic diversity of sunflower crop by accumulating, in a series of crosses between wild sunflower and crop lines, neutral diversity from seventy-seven wild sunflower accessions and the main already domesticated traits from three sunflower elite lines. During four successive cycles, we applied selection pressures both to improve the agronomical value of the population and maintain at its highest level the genetic diversity coming from the wild *H. annuus* compartment. After four generations of mixing in isolated plots, but always keeping separated the female progenies, we evaluated the diversity. We present here the experimental design, the characterization of the main agromorphological traits at each cycle and the analysis of response of important phenotypical and phenological traits in the HAS population as a consequence of the breeding constraints exerted on that genetic material.

MATERIALS AND METHODS

Plant Material

Seventy-seven *H. annuus* populations (HAS) were chosen among the set of 350 maintained at INRA Breeding station (Mauguio). They were screened for their spreading in the USA by covering most media under different climates and by the diversity of the morphological traits (Table 1). Between 1 to 8 populations were sampled in each of the following 15 US states.

Table 1. Distribution, by states, of the 77 collected accessions in North America

US State	<i>H. annuus</i> accessions with INRA CODE
ARIZONA	383; 386; 519; 654
CALIFORNIA	410; 421; 435; 437; 446; 458; 468; 833
COLORADO	363; 660; 980; 989; 996; 1147; 1148
ILLINOIS	211
IOWA	665; 829
KANSAS	733; 997; 998; 999; 1000
MISSOURI	351
MONTANA	943; 945; 948; 954; 1150
NORTH DAKOTA	928; 929; 931; 933; 939
NEW MEXICO	461; 463; 649; 661
OKLAHOMA	646; 651; 662
SOUTH DAKOTA	378; 388; 970; 1042; 1047; 1055; 1136
TEXAS	209; 509; 511; 647; 648; 650; 652; 734
UTAH	658; 774; 775; 822; 826; 1149
WYOMING	358; 361; 955; 963; 966; 974; 975

Construction of an intraspecific H. annuus genepool with wide genetic background

The wild and cultivated *H. annuus* were grown in the field at the INRA breeding station of Montpellier. Sowing dates were on 1 April 1996, in greenhouse with transfer in the field April 15 for G0, April 1st and 11 April 1997 for G1, April 1998 G2, May 17, 2006 G3, April 17 2007 G4. Five plants per population were grown in our nursery (G0) and each of the plants received separately pollen from the 3 testers, (branching allowed different crosses on the same plant). Testers were oil sunflower lines 89HR2, 90R19 and RT1B11. The two first are top branched (CMS-PET1 restorers) and the third is single headed (CMS-PET1 maintainer)

We grew 10 G1 plants per progeny (77 accessions x 3 testers x 10 individuals) the next year in a field isolated from gene flow from other sunflowers and we left the plants intercrossing. Each set of 10 plants was harvested separately and the seeds were bulked. At the next generation (G2) we grew up to 52 plants per progeny to enable a smooth elimination of too many branched individuals before they contributed to pollination. At the end of the G2 cycle, an average of 8 plants with reduced or no branching was harvested in each of the cultivated 228 progenies (3 combinations were missing). In order to induce fast increase for the domesticated traits and the agronomical value of the population, the following G3 and G4

generations were obtained after choosing the best individual in each progeny for maximum (OIL * 1000SW) combination value. Like in previous cycles, 5 plants per progeny were grown.

The measured variables

Each G0 plant was phenotyped for 13 morphological and phenological traits reported in Table 2.

Table 2. History of selection pressures applied on the wild *H. annuus* Pool (HAS), built on 1996

Cycle /year	Operation at Melgueil	Size of populations	Traits examined	Selection pressure
Cycle 0 (G0) 1996	F1 construction in field HAS= female parent 5 plants from each accession	77 HAS pollinated by 3 sunflower lines	Description origin, agromorphology + phenology + kernel size	None Harvest; only F1 hybrid kernels,
Cycle 1 (G1) 1997	Intermixing in isolation (=77 ecotypes x 3 testers x 10 plants)	228 F1 hybrid combinations with 10 plants per progeny 2280 F1 plants	Branching type intensity, leaf shape, pollen fertility, head diameter, earliness; kernel yield, oil content, TSW, seed yield.	Harvest: Whole plants ¹ * H1 (=10 plants separately) * H2, H3 (= 10 plants in bulk) G1 to G2: weak selection (24 % plant strongly branched were discarded for H1). None for H2 and H3
Cycle 2 (G2) 1998	Intermixing in isolation 228 maternal progenies (776 X 3 testers)	228 maternal progenies 11800 plants	Height + blooming date (start, medium, end) On harvested plants: Branching, high, TSW, oil content, Total W	Before blossom Elimination of most branched plants (bush form). At harvest: choice of 8 plants less branched. On the average, 84 % plants were eliminated in G2
Cycle 3 (G3-BL) 2006.	Intermixing in isolation (77 x 3 testers)	228 maternal progenies with 5 plants 1140 plants	Notations: blooming date, height, branching, TSW, oil content, seed yield	For each G2 progeny: the best OIL content * TSW plants were selected for G3. 80 % plants eliminated
Cycle 3 (G4-BL) 2007	Intermixing in isolation (77 x 3 testers)	228 maternal progenies with 5 plants 1140 plants	Notations: blossoming date, height, branching, TSW, oil content, seed yield	For each G3 progeny: the best OIL content * TSW plants were selected for G4. 80 % eliminated plants.

¹H1=89HR2, H2=90R19, H3= RT1B11

In the G1, G2, G3 and G4 generations the measured variables mainly concerned flowering period, branching, plant height, seed size, yield and oil content. Branching was defined according to two components (I) types of branching architecture including, basal, medium, apical, and (II) branching intensity scale related to the size of the branches. We used the grid shown in Fig. 1 to note the plants. Analyses were performed according to qualitative and quantitative notation grid presented in Table 3.

Table 3. Quantitative and qualitative characters measured on the HAS population through G0 to G4 generations

ACC	INRA code	Units / notation scale	HAS				
			G0	G1	G2	G3-BL	G4-BL
SOWFLO	Sow-flowering duration	days	X	X	X	x	X
HEIGHT	Plant Height	cm	X	X	X	x	X
BRAPIC	Basal branching	0: absent, 1: present	X	X	X	x	x
BRMED	Medial branching	0: absent, 1: present	X	X	X	x	X
BRBAS	Apical branching	0: absent, 1: present	X	X	X	x	x
NR	Unbranched plants	0: branched, 1: unbranched	X	X	X	X	X
INT_BR	Branching Intensity	0 (null) to 4 (high)	X	X	X	X	X
SW	Seed weight	g	X	x	X	x	X
TSW	1000 seeds weight	g	x	x	X	x	x
OIL	Oil content	%	x	x	x	x	x

















Type → Intensity ↓	Br_Basal	Br_Medium	Br_top	Br_Total (Br ↔ equi BMA)
0 Length. = 0 cm	← NR (Non ramifié) →			
1 Length. < 10 cm	 B1	 M1	 A1	 R1
2 Length. > 10 cm & < ½ Tige princ.	 B2	 M2	 A2	 R2
3 > ½ stem & < 1 main stem	 B3	 M3	 A3	 R3
4 > 1 main stem.	 B4	 M4	 A4	 R4

Fig. 1. Notation grid mixing ‘Type’ and ‘Intensity’ for branching of *HAS* and sunflower.

RESULTS

Construction and phenotypic variability in the HAS population

Our principle was to use enough HAS ecotypes to handle a genetic diversity representative of the HAS compartment in North America, to catch from the wild sunflower most of its favourable genes to enhance stress resistance and adaptation in sunflower and to drive the population to decrease the unfavourable traits such as branching, low oil content and seed size. As indicated in Table 4, an important variability is observed for the sow-flowering period among the plant population (more than 2 months), and plant height (from 71 to 308 cm). The branching was also characterised and all the wild *Helianthus* observed were extremely branched at all levels (apical, intermediate, basal), associated with high branching intensity. Average seed weight (TSW) was estimated at 9.9 g with a range of between 5.7 and 29 g. (close to cultivated types). On the other hand, average oil content of the 77 HAS was low (26.6%) and varied between 20.4 and 32.0%. Seed yield / head in open pollination varied between 0.1 and 9.5 g.

Table 4. Agro-morphological characteristics of the selected 77 accessions, in the G0 HAS population

Trait	Mean value	Std	Max	Min
Sow-flowering duration (SOWFLO), days	70.27	13.25	120.60	50.78
Plant Height (HEIGHT), cm	180.99	52.58	308.00	71.11
Max length of lateral branch (LAXL), cm	117.84	28.95	179.00	51.11
Basal branching (BRAPIC)	0.97	0.08	1.00	0.60
Medial branching (BRMED)	0.99	0.06	1.00	0.60
Apical branching (BRBAS)	0.97	0.11	1.00	0.20
Branching Intensity (INT_BR)	3.00	0.53	4.00	0.98
Total length lamina + petiole (LONGLEAF), cm	39.86	12.89	59.00	14.33
Petiole length (LONGPET), cm	17.70	7.26	4.22	4.22
Seed weight (SW), g	3.21	1.34	9.51	0.12
1000 seeds weight (TSW)	9.71	3.64	29.09	5.69
Head diameter (DHE), mm	29.24	6.23	68.64	17.81
Seed length (SLEN), mm	5.04	0.62	7.61	3.95
Seed width, (SWID), mm	2.47	0.30	4.04	1.99
Oil content (OIL), %	26.80	2.15	32.05	20.44

The G0 plants were evaluated for 15 traits in the Mauguio environment although they originated from different locations and climates in the USA. A PCA analysis (data not shown) gives the relations between measured phenotypic traits. Plotting of the geographical origin (State) on the graph provides identification of large geographical areas (northern, eastern, south –southwest US), grouping wild *H. annuus* with similar characteristics. Correlation analysis between main strengths of the climate

characteristics and morphology of the accessions showed that temperature was highly correlated ($r>50\%$) with height, leaf and petiole lengths.

Cycle length and plant height were significantly linked to precipitation ($r=0.4$) and temperature levels ($r= 0.28$ and 0.57 respectively). Additionally, plant height is connected to important rainfalls in short periods (coastal Texas climate). The apical and intermediate branching also seems to be affected by rainfall and to a lower number of rain days. Increased temperature range (maxi-mini) enhanced architectures with intermediate branching. The native accessions from geographical areas with a low rainfall (New Mexico, Arizona) were more branched. On the contrary, the different types of branching did not clearly respond to temperature.

Changes observed from G0 to G4 for main traits (Fig 2):

Changes in sowing-flowering period and plant height, plant branching, in the average oil content for seed size, seed yield were plotted. Although the experiments were not done the same year, we observed some general trends.

DISCUSSION

Systematic series of crosses between HAS and elite lines of domesticated sunflower have never been performed. We did not have any problems in the intercrosses, except some male sterility that segregated in some HAS. Here we reported several main features that tended to show that the material could be used for breeding sunflower and we tried to quantify our conclusions as much as possible. Some traits not retained in our notations were strongly selected such as seed dormancy, which was rapidly eliminated. Although HAS all displayed strong seed dormancy, we did not observe any problems in our progenies. This means that the trait is probably monofactorial and the locus efficiently eliminated. Branching behaved as a complex trait: we distinguished branching at 3 levels: basal, medium and top (Fig. 2). Basal and medium levels are unfavourable in the crop whereas top branching is used in most restorer lines. We surmised that the length of the branches was not inherited but due to the environment; the later the sowing, the shorter the branches for some lines and ecotypes. Non branching plants appeared in the G1 and due to selection pressure for the traits, it increased continuously. We used the grid (Fig. 1) to note the plants. We observed all the combinations of the elementary traits, which suggested that they are controlled by different genetic factors mostly independent. Seed weight was much lower in HAS (4-15g for 100 seeds) than in the crop (50-70g). However, when we chose the HAS we retained some with higher seed weight up to 30g. Two explanations are possible: 1) this is the range of variation for seed weight in HAS or, alternatively, 2) they have already been introgressed by domesticated sunflower. Due to the origin of the HAS with a large seed size in areas where the sunflower is cropped, we favoured the second hypothesis. However, in our experiment we used a combination of seed weight and oil content to avoid screening progenies with large seeds and poor oil content. Seed oil content did not increase rapidly, but we did not exert any strong selection pressure on the trait. Plant height decreased rapidly without any selection pressure. The variability is wide and height reduction will probably be rapidly feasible.

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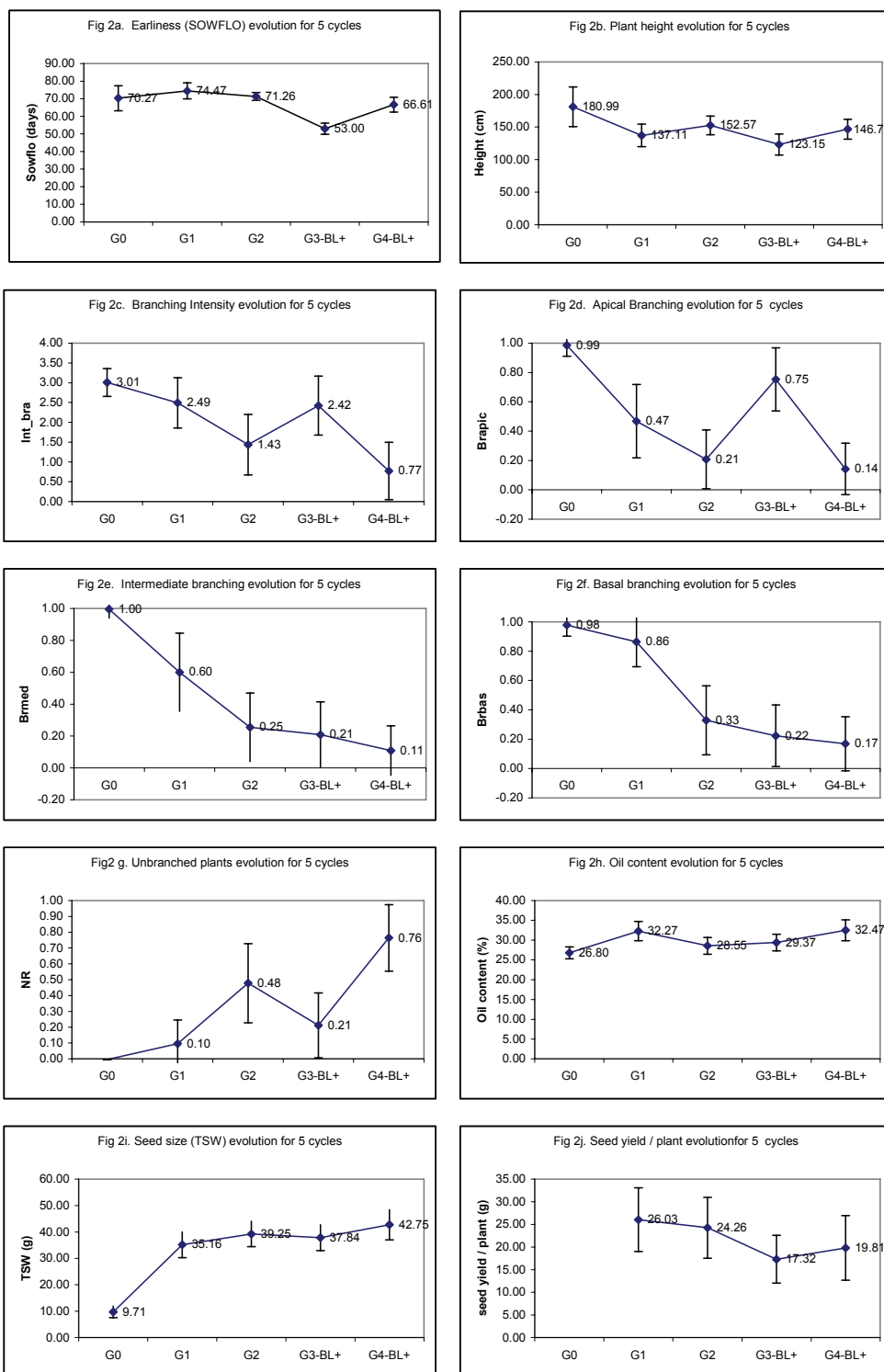


Fig. 2. Changes in phenological and morphological parameters along five generations in the neodomesticated progenies. SOBLO, sowing – blossoming stages in days. NR: non branching. Two G3 progenies were obtained in two different fields: One with ten plants per family with all 228 families (G3BL+), which has also led to G4BL+; and one with only 100 families (G3-Syspro)