

Assessment of interspecific hybridization between wild beet *Beta vulgaris* L. subsp. *maritima* and cultivated sugar beet under Moroccan conditions

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ABSTRACT

Obtaining a viable and fertile beet hybrid resulting from wild and cultivated sugar beet crosses under Moroccan conditions is challenging. To deal with hybridization, barriers in sugar beet, such as concordance of flowering period between biannual and annual subspecies, flowering aptitude according to vernalization requirement, genotype incompatibility and hybrid sterility, should be overcome. In our study, interspecific hybrids were obtained from crosses between 17 cultivated sugar beet *Beta vulgaris* subsp. *vulgaris* and 17 *Beta vulgaris* subsp. *maritima*, collected in different regions of Morocco. Twenty-two agro-morphological traits were used for the evaluation of 208 hybrid plants, under greenhouse conditions. CH and WH hybrids were collected on cultivated and wild parents separately. Most of the hybrids behaved as annual plants, of which 83.17% were able to flower and produce seeds without any vernalization requirements. All WH hybrids and 57.06% of CH hybrids were morphologically similar to their wild parent. However, 24% of CH hybrids were phenotypically similar to their cultivated parent. The remaining CH hybrid plants were behaving as intermediate genotypes between both wild and cultivated parents. Our results showed the success of interspecific beet crosses and emphasized the high variability among hybrid genotypes. This is of great importance for sugar beet improvement and breeding programs and interspecific crosses patterns understanding.

Keywords: genetic diversity, phenotypic characterization, vernalization requirement, heritability

INTRODUCTION

Gene flow or migration is the transfer of genetic material by interbreeding, from one individual to another of the same or genetically close species. The introduction of new genes through allo-polyploidization, interspecies hybridization, increases diversification within the population and leads to new combinations of traits, as well as the adaptability to new environments (Abbott et al., 2013; Soltis et al., 2015).

Beta vulgaris complex provides an excellent biological model of gene flow due to its cross-compatibility with its wild relatives (Panella and Lewellen, 2007; Biancardi et

al., 2020). Sugar beet (*Beta vulgaris* L.) is an economically important crop which accounts for almost 30% of the world sugar production (USDA-ERS, 2018). *Beta vulgaris* subsp. *maritima* (L.) Arcangeli is the main genetic resource of cultivated beet with a large ecogeographical and genetic diversity (Andrello et al., 2017). Agronomic traits introgression via interspecific hybridization between cultivated species and their wild relatives was carried out to confront biotic and abiotic stress, to improve yields in domesticated varieties and to enrich the genetic basis lost during domestication (Biancardi et al., 2012; Monteiro et al., 2018).

The existence of progenitors with different adaptive potential within the genus *Beta* enables the identification of potential hotspot of genetic diversity in order to enhance the tolerances to abiotic stresses, particularly in genotypes collected under extreme environments (Monteiro et al., 2018). These resources are also considered as drought and salinity tolerant plants (Panella and Lewellen, 2007; McGrath et al., 2011). The wild progenitors involved in interspecific hybridizations generate novel genetic diversity and can increase the adaptive potential.

Sea beet germplasms might provide an important reservoir of resistant genes to diseases and pests, that could be utilized in sugar beet breeding (Panella and Lewellen, 2007; McGrath et al., 2011). Recent major achievements in beet breeding relate to the identification of new sources of resistance and their introgression into sugar beet germplasm (Biancardi et al., 2010; Panella et al., 2016). These achievements were attained using multidisciplinary new technologies combining genetic and molecular tools. The identification of the Rz-genes on chromosome 3 (Capistrano-Gossmann et al., 2017), and their incorporation in sugar beet germplasms confer a resistance against beet necrotic yellow vein virus (BNYVV), which causes rhizomania, the major root disease of beet worldwide (Biancardi and Tamada, 2016). Furthermore, genetic resistance to *Cercospora* leaf spot (caused by *Cercospora beticola*) was more and less controlled (Taguchi et al., 2011). Cytoplasmic male sterility system (Owen, 1945) and doubled haploid sugar beet clones from ovule culture were used to develop hybrids for Rhizoctonia root rot and curly top resistance mapping (Eujayl et al., 2016).

In the Moroccan context where sugar beet is a relatively newly cultivated crop, interspecific hybridization is encountering numerous obstacles such as coincidence of the flowering period between an annual wild species and a biennial cultivated species, ability of flowering of sugar beet in limited vernalization areas, and the viability and adaptation of interspecific hybrids. To overcome these obstacles, enormous efforts have been made since 2015

within the INRA-Morocco Research Program to obtain viable and fertile hybrids, with a combination of genes of interest for cultivated beet germplasm enhancement and breeding.

In this context, the objectives of the present study are to: (i) succeed in interspecific crosses between sugar beet (*B. v. subsp. vulgaris*) and wild relatives (*B. v. subsp. maritima*) grown locally; (ii) evaluate morphological diversity within hybrids and between hybrids and their parents; (iii) establish heritability of studied traits.

MATERIAL AND METHODS

Plant material

Twenty-one interspecific hybridizations have been carried out between cultivated beet (*B. v. subsp. vulgaris*) and wild beet (*B. v. subsp. maritima*). Studied wild genotypes were collected during the reproductive period in 2010 and 2012 from areas with different geographical and ecological characteristics in Morocco. Sugar beet genotypes are originating from germplasm lines in the national breeding program. Cultivated genotypes involved in this study required a period of vernalization of 240 days to 261 days, from sowing to maturity, to flower and produce seeds. Table 1 summarizes the main ecological and behavioural characteristics of parental genetic material used in this study.

Methods

In October 2017, seventeen samples of cultivated beet *B. v. subsp. vulgaris* were transplanted after 1 month from sowing in the greenhouse at Merchouch, experimental station of the National Institute of Agronomic Research (INRA- Morocco) (33°36'48.8"N; 6°43'03.3"W; Altitude: 339 m). Experimental field is characterized by an annual cumulative rainfall ranging from 246.4 to 542.8 mm and a mean temperature reaching -0.5 °C in January and 45 °C in August during 2017 and 2018. This site was chosen for its adequate climatic conditions for vernalization and seed production of sugar beet genotypes.

Table 1. Agro-morphological and ecological characteristics of 17 wild and 17 cultivated beet parent genotypes involved in interspecific hybridization of Moroccan beet germplasm (*B. vulgaris* L.). WP: wild parent; CP: cultivated parent

	Parental genetic material	
	<i>B. v. subsp. maritima</i> (WP)	<i>B. v. subsp. vulgaris</i> (CP)
Vernalization requirement	Spontaneous (no requirement)	Cold period (-4 to 7 °C) for 45 to 70 days
Number of days to flowering (days)	145.9	240
Flowering time (days)	22.56	48
Seed formation period (days)	207.7	261
Average yield/plant (g)	4.18	133
Vegetal biomass	Low	Important
Life cycle	Annual	Biannual

Seventeen genotypes of *B. v. subsp. maritima* were planted under greenhouse conditions in December 2017, with a delay of 65 to 77 days compared to sowing date of cultivated genotypes, to synchronise flowering season between both parental forms.

Before flowering time, wild plants were transported to the field for the interspecific crossing, as shown in Figure 1. Parent genotypes were sown and characterized based on agro-morphological characteristics in 2016 - 2017, one year before the evaluation of interspecific crosses.

Two branches, one from each plant, were bagged with white parchment bags (12 × 18 cm) to control hybridization. At maturity, seed-balls were harvested

from both wild and cultivated branches separately so that maternity of each seed was known.

In December 2018, interspecific hybrids were sown under controlled greenhouse conditions with a temperature range between 22 and 28 °C and a humidity rate above 60%. A total of 208 viable plants were analysed in this study (Figure 2).

Morphological characterization

Twenty-two morphological traits were evaluated according to "Bioversity International" Beta descriptors (formerly International Plant Genetic Resources Institute: IPGRI, 1991).



Figure 1. Interspecific hybridization between cultivated beet "*B. v. subsp. vulgaris*" and "*B. v. subsp. maritima*" in Merchouch experimental station (INRA- Morocco). (1) Field trial with separate plots of cultivated sugar beet. (2) Hybridization with a bolting sugar beet plant in the field and a wild plant in the black hard plastic pots.

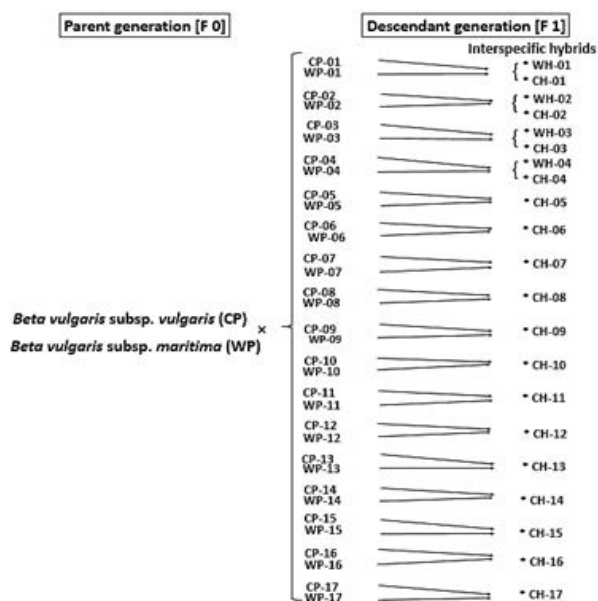


Figure 2. Crossing scheme of interspecific hybrids between 17 genotypes of *B. v.* subsp. *vulgaris* and 17 wild genotypes of "*B. v.* subsp. *maritima*". WH = hybrids from wild maternal branch. CH = hybrids from cultivated maternal branch.

Five qualitative traits related to leaf color (1 = Yellow ; 2 = Light green ; 3 = Green), petiole color (1= White ; 2 = Yellow ; 3 = Green ; 4 = Pink ; 5 = Red), stem color (1 = Green ; 2 = Green/Red ; 3 = Red) , leaf pigmentation (0 = Absent ; 1 = Spotted ; 2 = Red vein ; 3 = Entire red) and growth habit (1 = Erect ; 2 = Erect / procumbent ; 3 = Procumbent ; 4 = Erect/prostrate ; 5 = Prostrate) and seventeen quantitative traits related to leaf blade length (cm), leaf blade width (cm), petiole length (cm), petiole width (cm), plant height (cm), foliage diameter (cm), number of stems (main stem + side stems), germination rate (%), number of days to germination (from sowing to seedling emergence), number of days at bolting (from sowing to main stem elongation), number of days to flowering (from sowing to the start of flowering), flowering time (number of days from the beginning to the ending of flowering), number of days at seed formation (from sowing to seed formation), life span (from sowing to last harvest), number of seeds per plant (at the end of the vegetative cycle), weight of seeds per plant (g) and weight of 1000 seeds (g). These traits were related to vegetative and reproductive, developmental stages and yield. For the characterization of leaf surface, ten random leaves per plant were examined. The measurements were carried out on the plants before bolting.

Statistical analysis

As a means to characterize interspecific hybrids based on the morphological data sets, and to identify groups of phenotypically similar characters, statistical analyses were applied to both data traits sets separately.

Analysis of variance (ANOVA), and Duncan's multiple comparison test were used for quantitative traits. Principal Component Analysis was performed to determine the phenotypic proximity between hybrids and their parents. Linear regression coefficient in quantitative traits of hybrid plants was computed to obtain an estimate of broad sense heritability, as suggested by Hansche et al. (1972). Heritability analysis concerned wild parent (h^2_{wp}) and cultivated parent (h^2_{cp}). Two traits related to number and weight of seeds per plant were excluded from PCA and linear regression analyses, due to the clear difference between grain yield of cultivated and wild plants.

For the morphological qualitative characteristics, the frequency distribution of each morphotype was treated. All statistical analyses were carried out using SPSS software version 23.

RESULTS AND DISCUSSION

In the present study, seeds were harvested mainly on cultivated genotypes. Seeds were more abundant for 13 out of 17 crosses as follows (CH-05, CH-06, CH-07, CH-08, CH-09, CH-10, CH-11, CH-12, CH-13, CH-14, CH-15, CH-16 and CH-17). Four hybrid progenies were harvested both on wild (WH-01, WH-02, WH-03 and WH-04) and cultivated genotypes (CH-01, CH-02, CH-03 and CH-04). Results seem to lead to the hypothesis that cultivated beet were more receptive to wild pollen. Wild beet behaves more as pollinators than female plants for more than 83% of the crosses.

Genetic diversity of qualitative agro-morphological traits

With respect to qualitative traits, the results showed a large phenotypic variability in the beet progenies. Hybrid plants showed petioles with three colors (Figure 3, A). Green color proportion ranged from 50 and 78.6%,

red color proportion oscillated from 8.3 and 3.6%, and intermediate color between red and green was from 41.6 and 17.8% in WH and CH hybrids, respectively. Wild parents (WP) were characterized by four different petiole colors: green (32%), red (11%), green/red (47%) and pink (10%). However, uniform green petioles have been observed in all cultivated parents (CP). Green petioles observed in both parents may explain the high percentage of this morphotype in hybrid progenies, particularly in CH hybrids (78.6%). Red and red/green petioles were recorded in higher frequency in WH hybrids and were observed only in wild parent, suggesting the heritability of these morphotypes from wild maternal parent. Basically, CH hybrids tend to be similar to cultivated parents for the green-colored petiole (78.6%), in comparison with wild parent. In general, both WH and CH hybrids are situated at an intermediate level of petiole color between the parents.

Only green color blade was observed in all hybrid groups (WH and CH) as well as in their parents. This characteristic is transmitted to the progeny and remains stable for F1-generation.

Figure 3 (B) shows the results of qualitative traits related to stem color. Hybrid plants were characterized by green, red and green/red stems. Frequencies of these morphotypes recorded in WH hybrids were of 33.3% for green, 16.6% for red and of 50% for green/red petiole color. However, CH hybrids showed a percentage of 80.4% for green petiole color, 7.1% of red petiole color and 12.5% of green/red petiole color. These morphotypes were also observed in wild parent in a frequency of 5.26% for green, 84.4% for red and 10.52% for green/red stems. Cultivated parents were characterized by green stems. Therefore, red color is probably inherited from wild parent and transmitted to WH hybrids, in a higher percentage than to CH hybrids. Regarding the stem color, CH hybrids are more similar to cultivated parents 'CP', even if the 2 types of hybrid plants are intermediate between their parents.

Leaf pigmentation of each interspecific hybrid and parent genotype results are reported in Figure 3-C.

They show that leaves with a larger proportion of green pigmentation were dominant in the studied genotypes, explained by the absence of red coloration in the tissue. This color is due to the concentration and proportion of some red pigments, such as betalain and betanin in beet (Manetas, 2006; Hatlestad et al., 2012). Many scientific studies reported the evidence that betalains provided antioxidant and anti-inflammatory properties (Suganyadevi et al., 2010; Tan et al., 2015).

Low percentages of red veins (14.3%) and red spots (3%) on leaves were observed on CH hybrids, while CP parents have no red veins or spots. This trait is inherited from a wild parent and transmitted to both CH and WH hybrids. Figure 3-C shows that red veins are more expressed in WH hybrids (41.6%) compared to WP (21%). This result is probably due to the expression of hidden or recessive alleles in CP parents and expressed through the progeny.

Red pigmentation accumulation in leaves, stems and roots of betacyanic plants depends on the species (Cai et al., 2001) and is probably correlated with photoprotective functions of betacyanin in abiotic stresses (Burger and Edwards, 1996; Shu et al., 2009; Nakashima et al., 2011; Casique-Arroyo et al., 2014). In our study, WH hybrids presented higher red pigment accumulation in their tissue compared to CH hybrids. Genes coding for red pigmentation could therefore be transmitted from wild parent, characterized by a high resistance to biotic and abiotic stress (McGrath and Panella, 2018; Biancardi et al., 2020).

Phenotypic profiles related to growth habit show considerable variation. For the studied categories of beet (Figure 3-D), plants with erect and erect-procumbent growth habit were more predominant and observed commonly in all groups. Two other morphotypes (erect-prostrate and prostrate) have been present in low frequency ranging from 10.1 to 10.5% (erect-prostrate habit), and from 0.6 to 5.2% (prostrate habit), in CH and WP hybrids, respectively. Erect (33%) and erect-procumbent (67%) types were observed in equal proportions between WH hybrids and their cultivated parents indicating their

phenotypic similarity in growth habit. Furthermore, CH hybrids inherited two different and additional growth types, prostrate and erect-prostrate, from their wild parents.

With regard to the five studied morphological characters, qualitative phenotypic characterization of beet hybrids (F1) and their parental genotypes show great variability. In general, interspecific hybrids showed intermediate properties between cultivated and wild parents for most traits. They express maternal and paternal morphological characters in unequal frequencies, showing some heritability of these characters in progeny generation and the expression of new ones as a result of gene combination. Parental genotypes of *B. v. subsp. maritima* express more different morphotypes compared to cultivated ones. According to our study, these morphotypes appeared in the progeny probably explaining the considerable impact of the wild parent performance for the transmission of qualitative traits in interspecific hybridizations. In comparison to CH hybrids, a percentage of 91.66% of WH plant hybrids had inherited a more pronounced petiole red pigmentation (red/green: 71.6% and red: 8.3%), stems (red/green: 50% and red: 16.6%) and foliage (red vein: 41.6%). Wild parent contributed to the transmission of this trait to 13.37% of CH hybrids, even with small frequencies for spotted pigmentation (3%) and erect/prostrate (10.1%) and prostrate (0.6%) growth habit. 86.63% of cultivated parent issued hybrids (had mainly green and poorly pigmented foliage, similar to the dominant phenotypic appearance of cultivated parent).

Genetic diversity by quantitative agro-morphological traits

Characterization and variation study

The analysis of variance for quantitative phenotypic data showed statistically highly significant difference among the two studied interspecific hybrid groups WH and CH, except for traits related to petiole width (Table 2). Significant differences observed in different quantitative traits showed a genetic variation among the evaluated beet hybrid genotypes.

Coefficient of variation (CV) was calculated for each agro-morphological trait and for all tested groups of hybrids as well as their parents. A high variation was observed in the hybrids harvested on the wild parent branch, rather than those harvested on the cultivated one. CV overall ranges were between 0.55 and 214% for WH hybrids and between 0.29 and 122.6% for CH hybrids. In parallel, variation was higher in WP parents (17.33 to 116.9%) than CP parents (5.28 to 91.04%). A highly significant variability was revealed between cultivated parents and CH hybrids for leaf blade length and width, foliage diameter, and number of days to germination, to bolting, to flowering stages, and at seed formation. Significant differences within both parents were observed in plant height, number of seeds per plant, weight of seeds per plant and 1000-seeds weight.

In WH hybrids, the phenotypic traits related to leaf blade width, petiole length, foliage diameter, number of days to germination, to bolting and to flowering stages, seed formation and number of seeds per plant showed

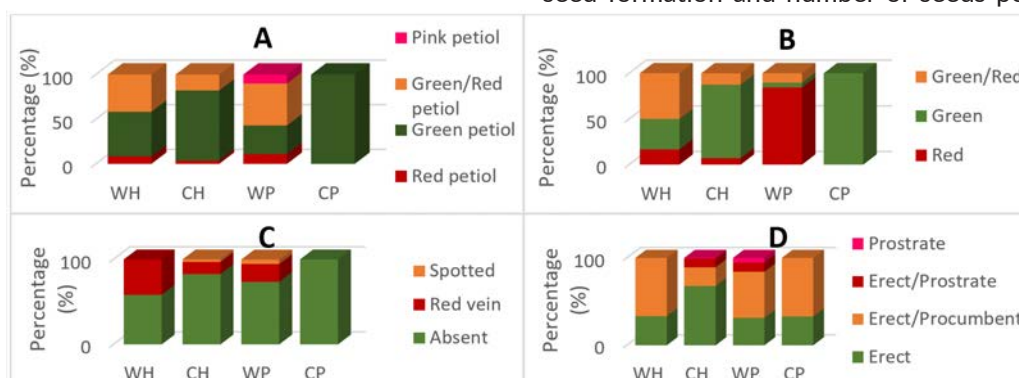


Figure 3. Qualitative traits related to petiole color (A), stem color (B), leaf pigmentation (C) and growth habit (D), used for the phenotypic characterization of, “WH/CH” hybrids obtained from crosses between *B. v. subsp. vulgaris* × *B. v. subsp. maritima*, as well as their wild parents “WP” and cultivated parents “CP”.

a significant difference between WH hybrids and their cultivated parents CP. These hybrids present significant differences in comparison to both wild and cultivated parents for four quantitative traits: leaf blade length, plant height, seed weight per plant and 1000-seeds weight per plant.

Mean values of hybrids and parent groups had a wider variation for different phenotypic traits (Table 2). Hybrids from cultivated maternal parents (CH), were characterized by an important vegetal biomass and late vegetative and reproductive stages. While hybrids from wild maternal parent (WH), showed relatively low vegetal biomass production and precocious developmental stages. Grain yield, number and weight of seeds per plant were also higher in CH hybrids, compared to WH hybrids, contrasting to 1000-seeds weight, which was slightly higher in WH hybrids.

Heritability and phenotypic similarity

The analysis of h^2 based on parent-offspring regression, and quantitative morphological data was used to estimate phenotypic relatedness between progeny and their parents. Histograms of h^2 based on R-square values of parent-offspring regression for 21 interspecific hybridizations are shown in Figure 4.

Estimated heritability ranged from 0.03 for petiole length to 0.98 for flowering time in wild maternal parents (h^2_{WP}), and from 0.02 for bolting to 0.91 for leaf blade length in cultivated maternal parents (h^2_{CP}).

In general, the studied traits were unequally heritable from parents. For phenotypic parameters related to plant growth in WH hybrids, observed phenotypic relatedness between hybrid plants compared to their wild and cultivated parent varied respectively from 0.05 for leaf

Table 2. Mean comparison of agro-morphological traits of interspecific hybrids, wild and cultivated parents of *Beta vulgaris* L.

Variables	WP	CP	WH	CH
Leaf blade length (cm)	12.19	16	6.73 **WP/**CP	11.14***CP
Leaf blade width (cm)	6.45	14	4.71***CP	7.2***CP
Petiole length (cm)	11.9	14	9.11 ^{CP}	11.25***
Petiole width (cm)	0.36	1	0.29	0.54
Number of stem (cm)	3.23	10	3.23***	4.48***
Plant height (cm)	77.63	119	59.85**WP/**CP	54.29**WP/**CP
Foliage diameter (cm)	39.41	60	34.06***CP	37.5***CP
Number of days to germination (days)	17.88	7	16.15 ^{CP}	18.26***CP
Number of days at bolting (days)	104.5	237	82.08***CP	129.08***CP
Number of days to flowering (days)	145.9	240	117***CP	151.3***CP
Flowering time (days)	22.56	48	13.38***	14.71***
Number of days at seed formation (days)	207.7	261	157***CP	177.9***CP
Life span (days)	210.4	309	203.3**	244.6**
Number of seeds/plants	168.6	2598	85.69***CP	196 ^{WP} ***CP
Weight of seeds/plant (g)	4.18	133	1.88***WP***CP	4.67***WP***CP
Weight of 1000 seeds (g)	18.18	22	13.14 ^{WP} ***CP	12.31***WP***CP
CV (%) interval	17.33 - 116.9	5.28 - 91.04	0.55 - 214	0.29 - 122.6

(*) significant difference depending on CP: cultivated parent/WP: wild parent (*P<0.05, **P<0.01, ***P<0.001) CV: coefficient of variation. WP: Wild Parent; CP: Cultivated Parent; WH: hybrids from wild maternal branch; CH: hybrids from cultivated maternal branch

blade length to 0.98 for flowering time, and from 0.07 for petiole width to 0.91 for leaf blade length. General trends observed in the heritability profiles of WH hybrids show that wild maternal parent has a predominant role in the transmission of evaluated characteristics. However, the leaf blade length was a heritable trait from the cultivated parent with h^2_{CP} ranging from 0.47 to 0.91.

The distribution of heritability rate values was asymmetrical for all quantitative traits measured the two parents in CH hybrid plants. For 208 genotypes of CH hybrids, the heritability estimation for traits related to plant growth ranged from 0.1 for petiole width to 0.89 for number of stems for h^2_{WP} . Concerning h^2_{CP} , the lowest value was 0.13 for petiole length and the highest value reached 0.89 for leaf blade length. The highest heritability rate of germination was between 0.54 and 0.89, and the lowest one was of 0.16 and 0.17 for h^2_{WP} and h^2_{CP} , respectively. Apart from interspecific crosses, CH-01, 02, 03 and 04 have remained in a vegetative state, without reaching the bolting and flowering stage. A total of 149 hybrid plants showed a moderate heritability ranging from $h^2_{WP} = 0.14$ (bolting) to $h^2_{WP} = 0.61$ (flowering time). h^2_{CP} values were fluctuated between 0.4 for bolting and 0.87 for flowering. The heritability h^2 estimates obtained for 1000-seeds weight ranged from 0.12 to 0.44 for h^2_{WP} , and from 0.17 to 0.79 for h^2_{CP} .

Moderate to high heritability was obtained in both parental genotypes. For most traits, a strong heritability rate (more than 50%) was observed for leaf blade length and width, plant height, foliage diameter, number of stems, germination, bolting, flowering, flowering time, seed formation life span and 1000-seeds weight with $h^2_{WP} \geq 50$ to 97%. For leaf blade length and width, petiole length and width, number of stems, foliage diameter, plant height, germination, flowering, flowering time, seed formation and 1000-seeds weight, heritability rate h^2_{CP} ranged from 55 to 91%. A low to moderate heritability was recorded for petiole width and length (h^2_{WP} from 10 to 48%), and for bolting and life span, $h^2_{CP} \geq 4$ to 40%. A dominant transmission rate of wild-parents phenotype was observed in 58 to 100% of WH hybrids for all

studied traits, except, the leaf blade length which present a heritable trait from cultivated parent in all WH hybrid plants. For CH hybrid plants, the cultivated parent is strongly involved in the transmission of phenotypic traits with a percentage of 12.5 to 85%, while up to 57% of CH hybrids showed phenotypic traits more or less similar to their wild parent. In addition, an intermediate phenotypic expression between the parents was also found in 6 to 53% of the CH hybrids.

Similar and heritable morphological traits have been identified in other species, as for plant height in *Festuca arundinacea*, the estimates broad-sense and narrow-sense heritability were high (83 and 97% respectively) (Majidi et al., 2009). In rice, Singh et al. (2007) also found a high heritability in broad sense recorded for plant height (62.86%), days to 50% flowering (93.25%) and grain yield (55.54%). Concerning sugar content in sugar beet, maternal impact was greater than paternal one. This result could be explained by progeny-mother heritability coefficient reaching 28.6%, substantially high compared with a progeny-father heritability coefficient of only 7% (Jassem et al., 2000). Heritability estimates based on parent-offspring regression is common and effective in beet breeding to improve white sugar yield and root yield, resistance to abiotic stress, such as drought tolerance, and biotic stress, such as resistance to *Cercospora* Leaf Spot (Smith et al., 1974; Setiawan et al., 2000; Ober et al., 2004; Würschum et al., 2013).

Phenotypic similarity between beet hybrids and their parents were demystified by a PCA conducted using quantitative traits related to plant growth, vegetative and reproductive developmental stages of 208 hybrids and 34 parent genotypes. Based on the first two axes PCA, hybrids and parent groups were divided into three main groups based on phenotypic quantitative traits (Figure 5).

The first group (blue circle) is the largest in terms of number of plants with 62% of total studied genotypes. 57.06% are CH hybrids (CH-05, 06, 07, 12, 13, 14, 15, 16 and 17), all WH hybrid plants and all wild parent (WP). This group was characterized by a low vegetal biomass, early developmental stages and a relatively high 1000-

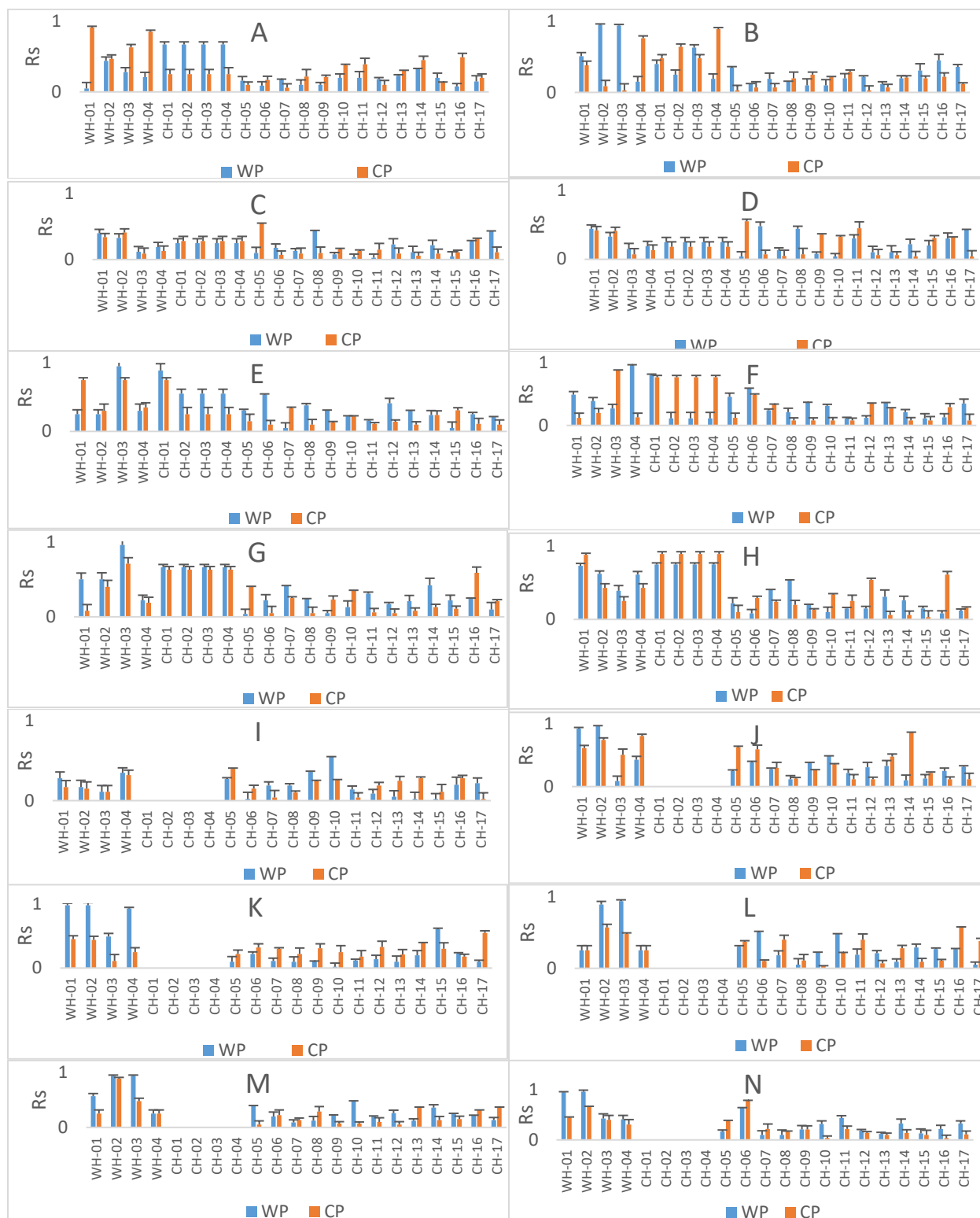


Figure 4. Histograms of h^2 based on R-square values of parent-offspring regression for fourteen quantitative traits of beet interspecific hybrids: (A) Leaf blade length, (B) Leaf blade width, (C) Petiole length, (D) Petiole width, (E) Number of stems, (F) Plant height, (G) Foliage diameter, (H) Number of days to germination, (I) Number of days at bolting, (J) Number of days to flowering, (K) Flowering time, (L) Number of days at seed formation, (M) Life span and (N) 1000-seeds weight. WP = wild maternal parent and CP = cultivated maternal parent.

seed weight (reaching 27.23 g). This result demonstrates the phenotypic relevance of most hybrids with WP parents, especially for leaf blade length and width, petiole width, plant height, life span and 1000-seeds weight.

The second group (green circle) concerned 21.15% of the total studied plants, with four CH hybrids (CH-08, 09, 10 and 11) and all cultivated parent (CP). This group was characterized by an important vegetal biomass and ramification, vegetative stages, late reproductive stages and a 1000-seed weight ranging between 9.7 and 13.23 g. A great phenotypic similarity was revealed between these four hybrids and their cultivated parents with the regard to leaf blade length, petiole size, number of stems, flowering date, development cycle to seed formation, as well as life span and 1000-seeds weight.

Similarly, the third group (yellow circle) included four CH hybrids (CH-01, 02, 03 and 04), representing 16.83% of total hybrid plants. This group revealed intermediate properties between wild and cultivated parents. Hybrids of this group didn't flower under greenhouse conditions. This could be explained by a requirement of vernalizing temperature with bolting resistant gene inherited from cultivated parent.

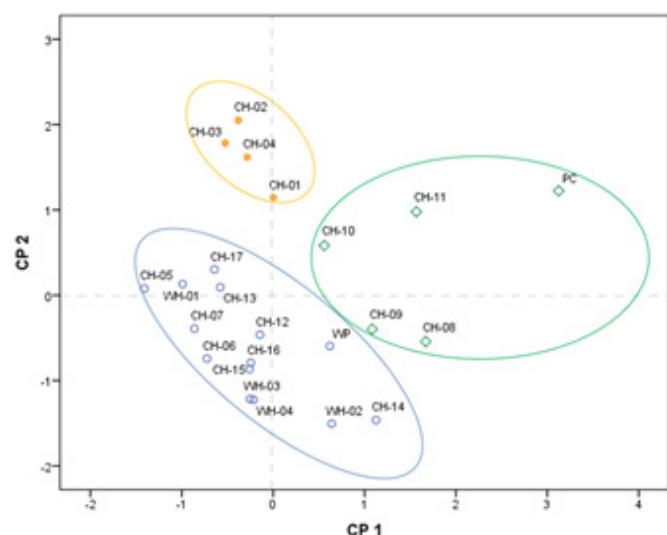


Figure 5. PCA of interspecific hybrid and parent groups based on fourteen phenotypic traits upon the first two axes, explaining 58.24% of the total variation. WP = wild parent of *B. v. subsp. maritima*, CP = cultivated parent of *B. v. subsp. vulgaris*, WH = hybrid from wild maternal branch, CH = hybrid from cultivated branch

Resultant PCA indicated a large variation in interspecific hybrid groups. This analysis is commonly used as an efficient tool to identify genetic diversity among the populations and genotypes in beet (Viard et al., 2004; Galewski and McGrath, 2020) as well as in several other species (Danbe, et al., 2018; Karagoz et al., 2020). Obtained hybrid characterizations revealed a broad phenotypic variation. It suggests that these genotypes could be used in beet breeding and genetic improvement programs, since the overlap of crossing barriers was achieved. A wide range of combined traits was revealed by the progeny.

Vernalization requirement

Flowering period synchronization between wild and cultivated beet is a major obstacle to interspecific hybridizations under Moroccan conditions. Therefore, obtaining a viable and fertile hybrid with a high germination rate exceeding 65% (Figure 6), remained a great success. This overlapping flowering periods requires a knowledge of locally adapted agricultural practices, especially a strict respect of flowering period coincidence between two parents. Cultivated sugar beet genotypes required a cold period of -4 to 7 °C for a period ranging from 65 to 77 days to induce bolting and flowering under Morocco climatic conditions. Many studies showed that cold period seems to influence the probability of flowering in beet and in other species (Van Dijk and Desplanque, 1999; Boudry et al., 2002). That has been mastered after several studies conducted by INRA.

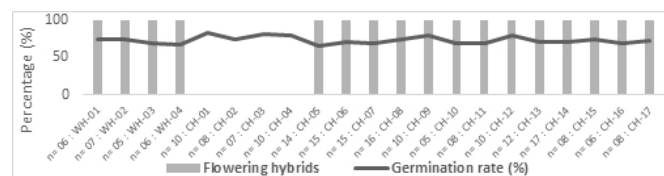


Figure 6. Germination rate (%) of 208 beet interspecific hybrids tested in greenhouse conditions. The histograms represent flowering hybrids. The number of genotypes investigated (n) is indicated.

The flowering has been shown to be largely requiring a vernalization period in cultivated *B. v. subsp. vulgaris* (Reeves et al., 2007). It is a process by which prolonged cold exposure can result in a transition from vegetative to reproductive stage. In most commercial cultivars of sugar

beet, flowering induction under long days (the threshold value being about 14 h, Van Dijk and Boudry, 1992) requires a cold period from 10 to 16 weeks of exposure to optimal temperatures between 4 and 6 °C (McGrath and Panella, 2018). The regulation of flowering time in beets is controlled by the interplay of two paralogs of two FT-like genes (*BvFT1/BvFT2*; Pin et al., 2010). Vernalization requirement appears to be strongly related to temperature (Limin and Fowler, 2006), geographical latitudinal gradients (Boudry et al., 2002; Rhoné et al., 2008), and photoperiod and cold treatment (Michael et al., 2020).

The absolute requirement for any vernalization is controlled by a single "bolting gene B" (Boudry et al., 1994; Hôft et al., 2018). Genotypes carrying the dominant B allele flower under long days without vernalization requirement (Van Dijk et al., 1997). A high percentage of studied hybrids (83.17%), including all hybrid plants from wild maternal parent of *B. v. subsp. maritima*, WH-01, 02, 03 and 04, and thirteen hybrids (CH-05, 06, 07, 08, 09, 10, 11, 12, 13, 14, 15, 16 and 17) from cultivated parent of *B. v. subsp. vulgaris*, flower under non-vernalizing conditions. While CH-01, 02, 03 and 04 (from cultivated parent) were not able to flower under the same conditions (Figure 6). The ability to flower of studied hybrids seems to be an inherited trait from wild parents carrying the allele B which cancels the vernalization requirement completely (Van Dijk et al., 1997; Boudry et al., 2002). The difficulty of flowering of 19.02% of CH hybrids could be explained by the impact of environmental factors such as high temperatures and light intensity, on Bb heterozygous genotypes. (Guan et al., 1992; Boudry et al., 1994).

A significant variability between and within interspecific crosses was observed for number of days to flowering compared to their cultivated parents. These fluctuated between 117 and 151 days in WH and CH hybrids, respectively (Table 2). Flowering time is an important characteristic feature in hybridization, where an overlapped and prolonged flowering time can increase the chances of crossbreeding between two parents. A non-significant difference in duration of flowering

was recorded in all tested hybrid groups. Flowering time was approximately the same among hybrids and varies between 13.3 and 14.7 days noted in WH and CH hybrids, respectively. However, a difference was observed in the flowering time for wild and cultivated parents, *B. v. subsp. maritima* (23 days) and *B. v. subsp. vulgaris* (48 days) (Table 2). Flowering synchronization as well as earlier reproduction in wild beets could be due to natural selection to ensure the perennity of the species (Boudry et al., 2002).

The timing of first reproduction in numerous plant species has been shown to be correlated with an interactive myriad of internal and environmental factors (Simpson et al., 1999), notably, day length, temperature, or vernalization requirement (Van Dijk et al., 1997; Limin and Fowler, 2006; Im et al., 2020). Under controlled greenhouse conditions, significant variability for seed formation (number of days to grain maturity) was observed among hybrids, with an average of 157 and 177 days in WH and CH hybrids, respectively (Table 2). The seed formation was observed in all WH hybrid plants and in 81% of CH hybrids.

The viable hybrids presented an intermediate behaviour between the two parents regarding the studied descriptive parameters. Globally, the tendency was towards an annual vegetative cycle. WH hybrids from the wild maternal branch are more similar to their wild parents for spontaneous flowering, indicating probably a pronounced genetic impact on vernalization requirement suppression (dominancy of B gene). That was also observed in CH hybrids from the cultivated maternal branch, however, only 19% of hybrids had a longer cycle approaching biennial cultivated beet and a high vegetal biomass.

The hybrids distinctly different from parental types were used for their high level of diversity that could produce new phenotypes. F1 progeny of different genotype crosses was also studied by several researchers to deal with the constraints and find possible ways to increase the level of sugar beet heterosis. Logvinov et al. (2019) reported that the seed productivity of simple

(single-cross) male sterile hybrids was on average 12.4% higher than the seed productivity of the original male sterile lines. The indicators of sterility and individual fertility have remained at stable level in individual single-crosses as well as in male sterile lines. In another study, heterosis in diploid and triploid F1 hybrids was not enhanced with the increasing of the paternal genome dosage and using highly heterozygous paternal genomes (Hallahan et al., 2018).

CONCLUSION

Obtaining a viable and fertile beet hybrid resulting from wild *B. v.* subsp. *maritima* and sugar beet crosses under Moroccan conditions is an important step for the development of sugar beet enhanced varieties. Beet hybrids showed considerable variation in interspecific crosses results, due to genetic combination between two different subspecies.

A moderate to high heritability was observed in most evaluated agro-morphological traits. In WH hybrids and a large proportion of CH hybrids, heritability from wild parents was relatively higher than from cultivated ones, making evidence of the relative genetic impact of wild parents on the progeny. Wild genotypes showed a higher performance of trait transmission in comparison with cultivated genotypes.

Agro-morphological traits were heritable from cultivated parent or from both parents in a less proportion of CH hybrids.

A high phenotypic diversity within and among hybrids was determined. Vernalization requirement can largely explain the differences in flowering between the hybrids. The majority behaved as annual plants, with an intermediate developmental stage between both parents. Hybrid that did not flower under non-vernalizing conditions during the first year, presented a longer cycle approaching the one of biennial cultivated parent, characterized by a high vegetal biomass.

Further investigation on the gene heritability and transmission based on obtained results will be for

considerable input for genetic diversity and germplasm enhancement, as the research is an initiation of the Moroccan wild beet germplasm genetic evaluation.

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