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RESEARCH ARTICLE

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Winter sowing of adapted lines as a potential yield increase strategy in lentil (*Lens culinaris* Medik.)

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Abstract

Lentil (*Lens culinaris* Medik. subsp. *culinaris*) is a traditional crop in Spain although current grain yield in Spain is relatively low and unstable. The effect of an early sowing date (winter sowing) on yield in the Spanish Central Plateau (*meseta*) was analyzed comparing it to the traditional spring sowing. Yield from eleven cultivars currently available for sowing in Spain and two $F_{6:7}$ populations of recombinant inbred lines (RIL), 'Precoz' × 'WA8649041' (89 lines) and 'BGE016365' × 'ILL1918' (118 lines), was evaluated in winter and spring sowing dates for three seasons (2005/06, 2006/07 and 2007/08) and two localities. Yield and stability were assessed by the method of consistency of performance with some modifications. When comparing with the best currently available cultivars sown in the traditional spring sowing date, (with an estimated average yield of 43.9 g/m in our experimental conditions), winter sowing using adapted breeding lines proved to be a suitable strategy for increasing lentil yield and yield stability in the Spanish *meseta*, with an average yield increase of 111% (reaching an estimated yield of 92.8 g/m). Results point to that lentil production can greatly increase in the Spanish *meseta* if adequate plant materials, such as some of the lines analyzed, are sown at late fall.

Additional key words: recombinant inbred lines; RIL; winter hardiness; yield stability.

Abbreviations used: B×I (cross 'BGE01636' × 'ILL1918'); CS (consistently superior); PE (Peñaflor de Hornija); P×W (cross 'Precoz' × 'WA8649041'); RIL (recombinant inbred line); ZA (Zamadueñas).

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Introduction

Lentil (*Lens culinaris* Medik. subsp. *culinaris*) is an annual cool season grain legume normally grown in temperate semi-arid regions, usually in rotation with cereals. It plays an important role in human nutrition. Lentils are traditionally valued as a source of proteins and iron, but also as an important dietary source of fiber, minerals, vitamins and antioxidants, in addition to carbohydrates, and its soil improvement properties, contributing to replenishing soil nitrogen levels (an average fixation of 80 kg/ha of which about 22 kg/ha remain in the soil) (Quinn, 2009; Pérez de la Vega *et al.*, 2011; Cokkizgin & Shtaya, 2013).

Lentil is a traditional food crop in Spain grown and consumed since the Neolithic (Buxó, 1997). It is cultivated mainly on the cold Central Plateau or *meseta*, characterized by cold winters and continental Mediterranean climate. Although the average grain yield in Spain is fairly low (680 kg/ha) with regard to other lentil producers, such as Canada (1,382 kg/ha), Turkey (1,433 kg/ha) or China (1,998 kg/ha), Spain is one of the most important lentil-growing countries of Europe: in 2013 it ranked first within Europe in production, 24,000 tons; area harvested, 40,000 ha, but 11th in yield (600 kg/ha), with an average production of approximately 18,000 tons/year. However, Spain imports about 48,000 tons/year (mainly from Canada and USA), 73%

of its needs (data from 2003–2012 average, FAOSTAT, 2015 (http://faostat.fao.org/). This means that a potentially interesting market is not covered by Spanish farmers, who neglect this crop because of its low economic profitability compared to rain-fed cereals (mostly barley), due to the low and unstable yield of lentil.

Barrios (2012), using an economic model based on historic yields and prices, and considering average production costs for lentil and barley (the most spread rainfed crop in the target region), concluded that the relative profitability between these crops in the Spanish Central Plateau would change if lentil yield increased by 30%. The definition of an agronomic strategy able to achieve this yield increase would constitute a good option for Spanish farmers to cover the interesting market now covered by imported lentils.

Spring is the traditional sowing date for lentil in the main area of cultivation in Spain. A good strategy to improve its yield would be switching to winter sowing. Winter-sown lentils benefit from cooler and more humid winter conditions. They make more efficient use of available moisture because the crop is established and growing when evaporative demand is minimal, and by avoiding heat stress and terminal drought at the end of growing season (Erskine & Akem, 1999). Increases in lentil yield due to winter versus spring sowing have ranged from 50% to 100% in several countries (Sakar *et al.*, 1988; Kahraman *et al.*, 2004a; Chen *et al.*, 2006). Siddique *et al.* (1998), in a study carried out in southern Australia, showed that seed yields greater than 1.0 t/ha and up to 2.5 t/ha can be achieved when sown early, and delayed sowing reduced yields by up to 30 kg/ha·day.

Early sowing requires cultivars well-adapted to winter conditions. Thus, lentil cultivars must have a phenology adapted to this cropping cycle, especially flowering time tuned to avoid stresses due to late spring frosts, but also winter hardiness to withstand winter conditions in cold areas, such as those of the Spanish *meseta* (Muehlbauer, 2004).

The aim of this research was to determine if winter sowing using adapted lentil materials would be a suitable strategy for increasing yield and yield stability in lentil in the Spanish *meseta*, and thus increasing its profitability, making it an attractive option for farmers in this region.

Material and methods

The populations used in this study were eighty-nine F_6 recombinant inbred lines (RILs) derived from the cross 'Precoz ' × 'WA8649041' (P×W), and 118 F_6 RILs derived from the cross 'BGE016365' × 'ILL1918' (B×I). A cold tolerant Syrian landrace ('ILL4400', Stoddard *et al.*, 2006), and eleven Spanish lentil cultivars, currently available to the Spanish farmers ('Águeda', 'Alcor', 'Aljama', 'Amaya', 'Ángela', 'Azagala', 'Candela', 'Guareña', 'Lyda', 'Magda' and 'Paula'), were included as checks.

The P×W population was kindly provided by Dr. F. Muehlbauer (USDA-ARS. Dept. Crop Soil Sci., Washington State Univ., Pullman, WA, USA). 'WA8649041' is a known source of winter hardiness, obtained from Turkish germplasm (Spaeth & Muehlbauer, 1991), with a prostrate plant structure, very strong branch habit, microsperma seeds with orange cotyledons, brown seed coat color and late flowering. The alternative parent, 'Precoz', is a macrosperma cultivar from northern Argentina (Erskine, 1983), moderately branched, semi-tall erect, yellow cotyledon, green seed coat colour and early flowering (Saxena, 2009). The population B×I was developed by the Area of Genetics of the University of León (Spain). 'BGE016365' is a landrace from Vega del Río Palmas (Las Palmas, the Canary Islands), susceptible to frost. The alternative parent 'ILL1918' is a lentil material from the ICARDA International Cold Tolerance Nursery and originated in Austria. Both are microspermas (although 'ILL1918' seeds are relatively large), moderately branched, semi-tall and erect, with brown seed coat color.

The RILs were sown at two locations in the province of Valladolid, (i) Zamadueñas experimental station (codified as ZA in tables and figures, 41° 39' 8'' N, 4° 43' 24'' W, elevation 690 m.a.s.l.) and (ii) Peñaflor de Hornija (codified as PE), 41° 42' 41'' N, 4° 59' 2'' W, 839 m.a.s.l), in 2005-06 (06), 2006-07 (07) and 2007-08 (08). Both locations mainly differ in elevation and agro-ecological conditions: PE is located in a bleak windy spot with colder winters and an average of 10 additional days of frost risk compared to ZA. ZA is located in a deep-soil river valley with higher yield potentials. For each *location* × *year* combination, two sowing dates were assessed: the first half of November (winter sowing) and the second half of February (spring sowing). In 2005-06 trials were only carried out in ZA because of limited seed availability. Due to a plague of common voles (*Microtus arvalis* L.), 2006-07 PE trials were excluded. Thus, finally there were data available from eight environments considering *sowing date* × *location* × *year* combination. The average growing cycles were of 234 and 157 days for winter and spring sowing, respectively. Plants were harvested after full maturity, at the end of June.

For each environment, the experimental design was a randomized complete block design with three replications. The experimental unit was a plot consisting of a single row 2-m long sown with 40 seeds, similar to the experimental units considered by Kahraman *et al*. (2004a,b) in their lentil RILs field trials. The rows were spaced 0.33 m. Conventional agronomy practices carried out by farmers in the area for tillage, fertilization, weeds and pest management were applied (Barrios, 2012). Yield was calculated as the seed weight after hand harvesting and threshing of each experimental unit, expressed as g/m (grams harvested per each lineal meter of row).

Environmental, genotypic effects (fixed factor) and *genotype × environment* interaction were analysed by analysis of variance, considering location/year (random), sowing date (fixed) and their interaction for partitioning the environmental source of variation. Genotype potential and adaptation to sowing date were analyzed considering yield genotype superiority and stability indexes according to the method of consistency of performance (Ketata *et al.*, 1989) with some modifications based in the stratified rank method proposed by Fox *et al.* (1990), considering three different environmental combinations: the whole set, and the winter and spring ones separately. Based on this analysis, for each RIL population and check cultivars set, consistently superior genotypes for winter and spring sowing dates were identified, and a multiple comparison test for yield least squared means was carried out using the Tukey–Kramer HSD method.

Results

Analysis of variance detected differences with an associated probability below 0.01 for all the sources of variation considered. *Genotype by environment* interaction accounted the highest coefficient of determination, explaining 49% of the variability included in the model, whereas environment and genotype explained 30% and 20%, respectively. Sowing date was the most influencing environmental source of variation, explaining 51% of the environmental variability. The interactions involving sowing date represented 58% of the G×E interaction (Table 1).

The consistency of performance considering RIL lines, population parents and lentil checks, for the whole set of environments and the winter and spring ones, is shown in Figs. 1A, B, C, respectively. Among the RIL parent genotypes, 'Precoz' and 'WA8649041' showed opposite yield rank in all three graphs, although yield stability was always high. On the other hand, 'BGE016365' and 'ILL1918' showed different and generally intermediate positions in both rank and stability.

Most of the checks cultivars were not included in the quadrant representing consistently superior genotypes (lower-left in Fig. 1A to 1C), except 'Águeda', 'Paula' and 'Azagala', that showed high and stable yield in spring-sowing environments (Fig. 1C), so they would be considered as the best yielding and adapted check cultivars for the traditional spring sowing date in the target region.

Five RILs from P×W and 17 RILs from B×I were identified as consistently superior genotypes for the whole set of environments (Fig. 1A). Twenty-one RILs (10 from B×I and 11 from P×W) and 19 RILs (14 and 5 respectively) were respectively included for the winter sowing (Fig. 1B) and spring sowing (Fig 1C) environments. Five RILs, B-79, B-117, B-137, W-33 and W-40, were consistently superior for both winter and spring environments (Table 2), thus constituting prom-

Table 1. Analysis of variance of lentil yield considering environment, genotype and genotype by environment interaction as sources of variation and their partition including sowing date (spring *vs.* winter).

Source of variation	d.f.	RSq	P>F
Model	1799	0.97 [1]	< 0.01
Env	7	0.30 [2]	< 0.01
LocYea	2	0.25 [3]	< 0.01
SDate	1	0.51 [3]	< 0.01
LocYea * SDate	3	0.24 [3]	< 0.01
Geno	222	0.20 [2]	< 0.01
Geno * Env	1554	0.49 [2]	< 0.01
LocYea * Geno	666	0.42 [4]	< 0.01
SDate * Geno	222	0.20 [4]	< 0.01
LocYea * SDate * Geno	666	0.38 [4]	< 0.01

d.f.: degrees of freedom; RSq: coefficient of determination; Env: environment; LocYea: location/year combination; SDate: sowing date; Geno: genotype; *: interaction. [1]: from total; [2]: from model; [3]: from environment; [4] from genotype * environment interaction.

Table 2. Lentil genotypes identified using the consistency method included in the higher and more stable yield[1] quadrat considering spring and winter sowing environments.

	Spring	Winter
Parental lines	**Precoz**	ILL1918, **Precoz**
B×I RILs	14, 54, 67, 77, 78, **79**, 88, 93, 102, **117**, 120, 127, 128, **137**	10, 40, 41, 71, **79**, 107, 113, **117**, **137**, 147
P×W RILs	**33**, **40**, 56, 58, 83	15, 16, 32, **33**, 36, 37, **40**, 73, 80, 88, 91
Checks	Águeda, Azagala, Paula	—

[1] Genotypes located at the lower right quadrant (next to 0.0 point) of each graphic in Fig. 1. B×I: 'BGE016365' × 'ILL1918'. P×W: 'Precoz' × 'WA8649041'. High and stable yield genotypes in both environments are indicated in bold.

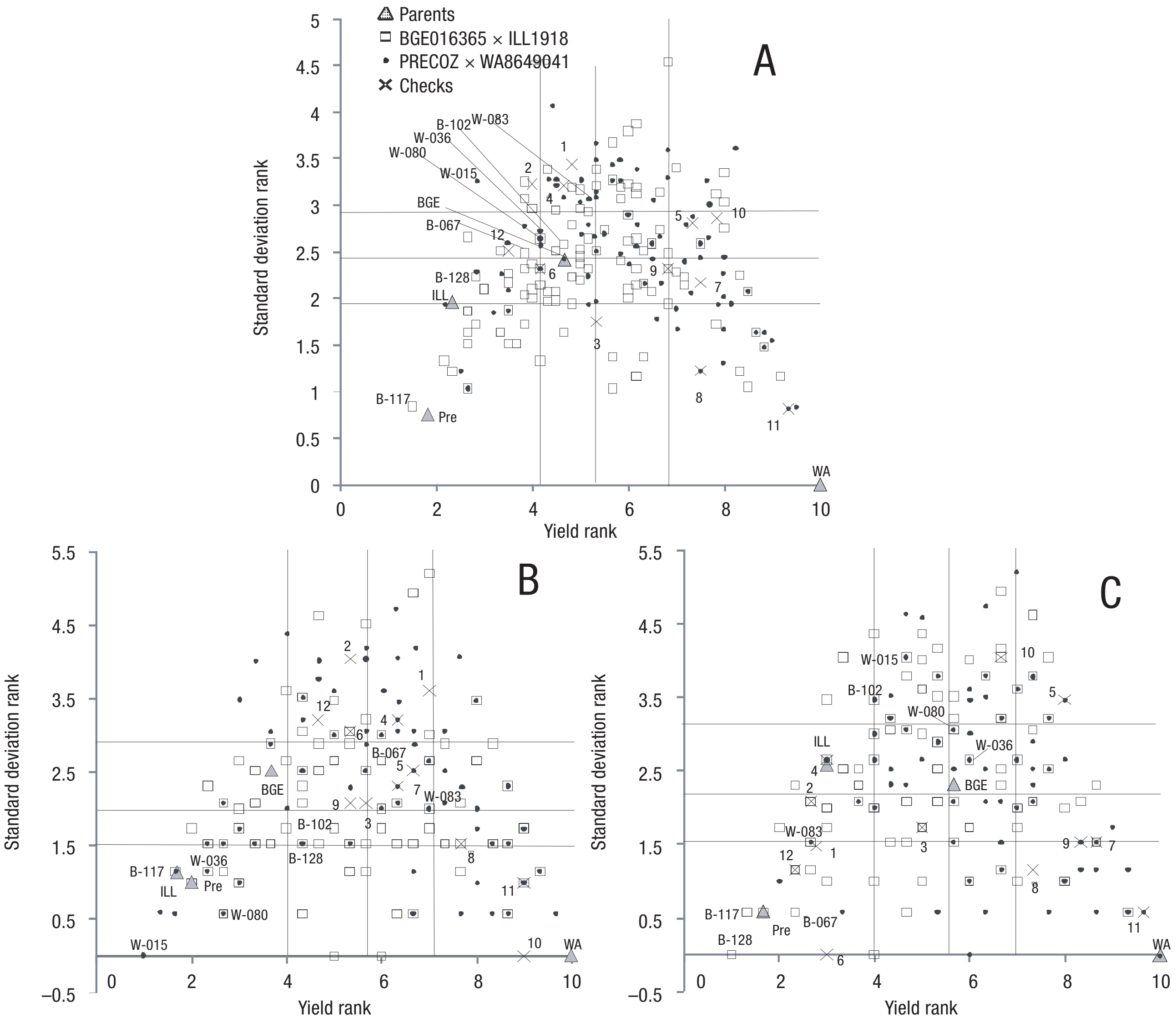

Figure 1. Consistency of performance test derived plots considering all environments (A), the winter sowing environments (B), or the spring sowing environments (C). Lentil RIL parents are indicated by triangles as follows: BGE = 'BGE016365', ILL = 'ILL1918', Pre = cv. 'Precoz', WA = 'WA8649041'; RIL lines are indicated by squares ('BGE016365' × 'ILL1918') or dots ('Precoz' × 'WA8649041'), respectively; lentil cultivars used as control are indicated by X; 1, 'Águeda'; 2, 'Alcor'; 3, 'Aljama'; 4, 'Amaya'; 5, 'Ángela'; 6, 'Azagala'; 7, 'Candela'; 8, 'Guareña'; 9, 'ILL4400'; 10, 'Lyda'; 11, 'Magda'; 12, 'Paula'. Some particular RIL lines are highlighted by comparative purposes, those from 'BGE016365' × 'ILL1918' are indicated as B and those from 'Precoz' × 'WA8649041' as W. Note: Some points in the graph include more than one RIL line.

ising breeding material combining yield potential and general adaptation to sowing date.

The best checks ('Águeda', 'Azagala' and 'Paula', Table 3a) did not show significant increment of yield due to winter sowing when considering the whole set of environments (even they showed a significant -70% decrease in ZA08). This result suggests that winter sowing of currently available lentil cultivars would not be a good strategy to increase lentil yield in the target region.

Although the total average increment of both winter and spring adapted RILs sets from B×I (B×I W-CS and B×I S-CS) due to winter sowing was significant (84% and 36% respectively), this was not true for ZA08, likely because in this trial the rainy conditions during the spring favored equally grain yield for both sowing dates. For the spring adapted RIL set from P×W (P×W S-CS, Table 3a), winter sowing significantly increased yield in two combinations, Z07 (90%) and P08 (49%), but was not significant for the average results. For the group of RILs selected for winter adaptation from P×W (P×W W-CS), in all the location/year combinations considered, winter sowing significantly increased yield with respect to spring

Table 3. Tukey-Kramer HSD test comparisons for yield increase (%) considering selected RILs and the best checks under spring and winter sowing conditions.

A: Winter vs. spring sowing yield increase (%)

Genotype group	Nº genotypes	ZA06	ZA07	ZA08	PE08	Total
Best checks	3	**106.3** *	**193.8** *	-69.8 *	10.1	25.8
B×I S-CS	14	**90.8** *	**143.7** *	-30.4 *	**69.2** *	**36.4** *
P×W S-CS	5	18.5	**90.1** *	3.5	**49.4** *	29
B×I W-CS	10	**152.3** *	**290.4** *	-14.4 *	**157.6** *	**84.0** *
P×W W-CS	11	**158.4** *	**290.8** *	**126.4** *	**168.0** *	**169.2** *

B: Yield (g/m) and yield increase (%, between brackets) *vs.* spring sowing of best checks

Sowing date	Genotype group	ZA06	ZA07	ZA08	PE08	Total
Spring	B×I S-CS	39.4 (-0.2)	21.9 (-3.1)	**89.8** * **(38.2)**	46.5 (-4.5)	49.4 (12.5)
	P×W S-CS	42.9 (8.6)	23.4 (3.7)	72.4 (11.4)	41.8 (-14.2)	45.1 (2.7)
	B×I W-CS	33.8 (-14.3)	16.7 * (-25.9)	**85.5** * **(31.5)**	35.9 * (-26.2)	43.0 (-2.3)
	P×W W-CS	33.3 (-15.7)	19.7 (-12.7)	48.0 * (-26.2)	37.1 * (-23.8)	34.5 (-21.5)
Winter	B×I S-CS	**75.2** * **(90.4)**	**53.4** * **(136.1)**	62.5 (-3.8)	**78.7** * **(61.6)**	**67.4** * **(53.5)**
	P×W S-CS	**50.8** * **(28.7)**	**44.6** * **(97.2)**	**74.9** * **(15.3)**	**62.4** * **(28.2)**	58.2 (32.6)
	B×I W-CS	**85.4** *a **(116.2)**	**65.4** * **(189.4)**	73.1 (12.5)	**92.6** *a **(90.1)**	**79.1** *a **(80.1)**
	P×W W-CS	**86.0** *a **(117.8)**	**77.1** *a **(241.2)**	**108.6** *a **(67.1)**	**99.4** *a **(104.1)**	**92.8** *a **(111.3)**
Yield (g/m) for best checks in spring sowing date		39.5	22.6	65.0	48.7	43.9

PE: results from Peñaflor de Hornija location; ZA: results from Zamadueñas location; 06, 07, 08: 2005-2006, 2006-2007, 2007-2008 trials respectively. Best checks: Águeda, Azagala and Paula cultivars; B×I: RILs selected from BGE016365 × ILL1918; P×W: RILs selected from 'Precoz' × 'WA8649041'; S-CS: RILs defined as consistently superior (CS) for spring sowing environments; W-CS: RILs defined as consistently superior for winter sowing environments; *: Significantly different according Tukey-Kramer HSD test (p<0.05). In bold: significantly higher yield. a: Highest yielding *genotype group* × *sowing date* combination (and not significantly different combinations to it), according to Tukey-Kramer HSD test (p>0.05).

sowing 126 to 291%, with a total average increment of 169%, the maximum detected.

When compared against the yield of the best lentil checks in the traditional spring sowing conditions, none of the best adapted RIL sets (B×I and P×W) showed a significant yield increase under spring sowing conditions (W-CS even showed a significant lower yield in some year-locality combinations) (Table 3b). This means that the traditional spring sowing date would not grant better results of the new RILs as lentil crop in the target region.

In contrast, no significant reduction in yield was observed for winter sowing of the selected RILs (Table 3b). The total average yield increment was significant for both B×I selected sets (80 and 53% for winter, B×I W-CS, and spring, B×I S-CS, respectively). Again, the exception to the significant yield increment was the combination ZA08.

For both P×W selected sets (P×W S-CS and P×W W-CS), winter sowing resulted in a significantly better option than spring sowing of check cultivars for all the location/year combinations, with a yield increase ranging from 15% to 97% in the case of the spring adapted set and from 67% to 241% for the winter adapted one, with an average increase of 111%. In addition, the P×W winter adapted selected RIL set sown in winter was the highest yielding option for all the location/year combinations and for the total average results (Table 3b).

Discussion

The parental lines showed differential behaviors in different sowing dates. 'BGE016365' showed intermediate yield and yield stability in all conditions. This landrace comes from a mild climate region, and is probably poorly adapted to the harsher conditions of the *meseta*. 'ILL1918' showed good production with better stability in winter than spring sowing. More noticeable is the behaviour of the two other parents. While 'Precoz' was among the highest yielding materials, 'WA8649041' showed a poor yield under all conditions (Fig. 1). 'WA8649041' is a known source of winter hardiness (Spaeth & Muehlbauer, 1991); however, it showed a very poor adaptation to the Spanish *meseta*, even under winter sowing conditions. The most likely reason for this low yield is the long life cycle, with a late start of flowering date which likely caused the observed flower abortion and affected seed filling as a consequence of heat stress and terminal drought. On the other hand, 'Precoz', although it was described as a material with poor winter-hardiness (Kahraman *et al.*, 2004a,b), showed good adaptation to the winter conditions of the Spanish *meseta*, as its short life cycle likely helped to avoid heat stress and terminal drought. These results suggest that winter hardiness is a very complex trait highly dependent on the differential, changing and somehow unpredictable winter conditions (Muehlbauer, 2004). Further studies on individual characters and specific yield components would explain the different yield potential and adaptation patterns detected in the RIL progenies.

Most of the lentil cultivars currently sown in Spain proved to be better adapted to spring sowing conditions, thus justifying their use in such traditional conditions in the *meseta*, but only three of them were included among the best yielding materials in these sowing conditions (Fig. 1C).

In general, the observed effect of the sowing date on lentil yield in the Spanish *meseta* agrees with previous studies in which an earlier sowing date increases lentil yield, both in Mediterranean climate zones (Fagnano *et al.*, 1998; Siddique *et al.*, 1998; Chen *et al.*, 2006), or in other climates (Kusmenoglu & Aydin, 1995; Hamdi *et al.*, 1996; Muehlbauer & McPhee, 2002; Sarker *et al.*, 2009). But, according to our results, this effect would depend on the genotypes considered. Thus, to achieve the desired increase in yield, not only a shift in sowing date would be required, it must be coupled with a proper varietal choice.

In our trials, the combination of changing the sowing date to winter together with the use of winter adapted lentil material increased the potential average yield. For instance, winter-adapted RILs derived from P×W would almost duplicate (111%) the yield obtained from the three best currently cultivars sown in the traditional spring sowing date (Table 3). This potential increment in lentil yield is clearly superior to the 30% proposed to reach similar profitability between lentil and barley in the region (Barrios, 2012). Although comparisons carried out in this type of trials need to be checked in real farmer plots and management, it seems evident that the agronomic strategy analyzed here is a promising alternative to attract the attention of farmers for this crop and a way for them to supply the interesting lentil market in Spain.

As a main conclusion, this work contributes strong evidence that lentil production can greatly increase in the Spanish *meseta* if adequate plant materials are sown at late fall, and also detects pre-breeding materials available to accomplish this goal.

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