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Capturing hybrid vigor for lentil breeding

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Abstract

Conventional breeding has produced low levels of yield increase in lentils (*Lens culinaris* ssp. *culinaris* Medik.) in recent decades. As hybrids have led to large increases in yield in other crops, we examined the possibility of using hybrids in lentils. In 52 hybrids between Australian commercial cultivars and lines from a global collection of cultivated and wild accessions, we found 15 of the hybrids had seed yield hybrid vigor, with better parent heterosis (BPH) gains ranging from 10.4 to 125%. Flowering time, seed size, cotyledon color, and branch number were important but not critical for the level of hybrid vigor in seed yield. Plant height at 28 days post sowing (dps) could be an indicator of seed yield vigor of hybrids grown in glasshouse conditions. In the absence of male sterile lines in lentils, we used high-yielding hybrid combinations in a program of repeated selfing and selection to produce stable, high-yielding lines (Hybrid Mimics) with grain yields similar to the F1 hybrids, avoiding the limitation of the hybrid advantage to the F1 generation. Seed yield increased in successive generations (F3–F5); yields of the F4 plants were close to that of the F1 hybrid. Our results indicate that there is potential for hybrid seed yield vigor in lentil to be accessed through hybrid mimic selection. This is a breeding method not dependent on male sterility that can be initiated with a low number of hand-pollinated hybrids. Hybrid mimic breeding may be applicable to a wide range of crops.

1 | INTRODUCTION

As one of the first domesticated crops, lentil (*Lens culinaris* ssp. *culinaris* Medik.) ranks fifth in the global production of grain legumes (Joshi et al., 2017). It is a nutritious, staple food, rich in protein (~30%), high in fiber, folic acid, magnesium, and iron but low in fats. It is the main protein source in many South Asian countries, especially in the Indian subcontinent. Lentil is a dryland crop and can be grown under water-limited, rain-fed environments. Lentil can fix nitrogen and is useful as a rotation crop in agricultural systems in a number of semi-arid regions. Lentil grains have cotyledon color differences regulated by a single locus, *Yc*, which is located close to a SNP marker LcC13114p356 (Fedoruk et al., 2013; Slinkard, 1978). Red lentils (with orange cotyledon color) and green lentils (with yellow cotyledon color) have different markets. The production and consumption of lentils have increased in recent decades with Canada and India having major roles in the world market. In other regions, such as Europe and Australia, lentil is an important crop. With an increasing global population and increasing demand for plant protein, lentil is likely to play an expanding role in global food security.

Abbreviations: AGG, the Australian Grains Genebank; BPH, better parent heterosis; dps, days post sowing; MPH, middle parent heterosis; MPV, middle parent value.

The genus *Lens* consists of seven taxa grouped into four species. *Lens culinaris* ssp. *orientalis* is the most closely related wild progenitor of the cultivated lentil (*L. culinaris* ssp. *culinaris*), and *L. nigricans* is the most distant relative (Wong et al., 2015). The self-pollinated crop (*L. culinaris*) is diploid with a relatively large genome size ($2n = 2x = 14$, ~4.3 Gigabytes). A pre-release draft genome sequence has been available since 2016 from the International Lentil Genome Sequencing Initiative (https://knowpulse.usask.ca/Lens/culinaris). Lentil, cultivated since 8,000 B.C., is thought to have little genetic diversity. Data from FAO (www.faostat.fao.org) showed <2% annual yield increase. A recent analysis of 19 Australian cultivars released between 1988 and 2019 showed 1.23% per year of seed yield gain. Similar results were obtained in Canadian cultivars (Sadras et al., 2021).

Low genetic diversity was considered to be one of the main barriers to yield increase (Foyer et al., 2016). Genomic and genetic studies have been carried out in lentil (Bermejo et al., 2020; Khazaei, et al., 2018; Ogutcen et al., 2018). Wild germplasm has been used as a resource to broaden the genetic base of cultivated lentils (Gorim & Vandenberg, 2018; Singh et al., 2018). *Lens culinaris* ssp. *orientalis* and *L. ervoides* produce fertile hybrids when crossed as a pollen donor with cultivated lentils (Singh et al., 2017). Crossbreds have been used in lentil breeding and have shown the potential for yield increase. A study of 19 diverse lentil genotypes, including material from South Asia and West Asia, and their crossbreds, showed the seed yield of crossbreds, South Asian and West Asian genotypes were 1,550, 1,270, and 330 kg ha^{-1}, respectively (Shrestha et al., 2005). The more than 20% yield increase in crossbreds indicated the promise of hybrids in breeding programs.

Hybrid breeding, using the marked yield increase of the F1 generation relative to parents, is widespread in maize (*Zea mays* L.), rice (*Oryza sativa* L.), and other crops (Duvick, 1999). In Arabidopsis and rice, hybrid vigor is correlated with early germination, which has an effect on the F1 plant at an early stage (He et al., 2021; Zhu et al., 2017). The vigor of the F1 hybrid is restricted to a single generation. In legumes, hybrids with increased yields of 50% or more relative to parents have been reported in pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), common bean (*Phaseolus vulgaris* L.), cowpea [*Vigna unguiculata* (L.) Walp.] and chickpea (*Cicer arietinum* L.) (Ali et al., 2013; Bakhsh et al., 2007; Bhawna & Singh, 2015; Sarawat et al., 1993; Saxena et al., 2021; Stelling, 1997). The first commercial pigeonpea [*Cajanus cajan* (L.) Huth] hybrid was released in 2013 (Saxena et al., 2013). Hybrid breeding has rarely been discussed in other legumes, probably because of the difficulty of developing hybrids, especially for crops such as lentil where pollination occurs in small unopened flowers and there are no male sterile lines to use in large-scale production of hybrid seed (Saxena-et al., 2021). When hybrids can be produced they can only be used for one generation as selfing of the F1 leads to segregation of traits producing a crop that is heterogeneous in phenotype and with yield decreased from that of the F1 hybrid.

Core Ideas

- Hybrids provide new opportunities in lentil breeding.
- Heterosis of seed yield was found in many lentil hybrids.
- High-yielding, stable lines (Hybrid Mimics) can be selected from selfed hybrids.

Recently in Arabidopsis and rice the increased yield of the F1 hybrid was able to be continued in subsequent generations using Hybrid Mimics where offspring with F1 hybrid-like high levels of biomass and seed yield were selected from the F2 and subsequent generations (He et al., 2021; Wang et al., 2015). High performing stable lines with characteristics similar to the F1 hybrids were obtained in the F5 and subsequent generations. Our aim was to develop a method for lentil improvement by determining if hybrid vigor exists in lentil and whether it could be captured in stable lines as Hybrid Mimic cultivars.

2 | MATERIALS AND METHODS

2.1 | Plant material

We collected parental material with high genetic and geographic distances between accessions. The initial material was obtained from Pulse Breeding Australia (http://www.pulseaus.com.au/). Four representative commercial cultivars were selected; Hurricane, Nugget, and Jumbo2 have small, medium, and large red cotyledon seed, respectively; Greenfield has a medium-sized green seed. To increase the gene pool, we also collected material from the Australian Grains Genebank (AGG), which includes both breeding collections and wild accessions (*L. orientalis*) from worldwide location. Two green lentil lines, large green-Pelagonia and small green-Dupuy, were collected from local stores. A pedigree of the origin of our material has been prepared with the limited information available (Supplemental Figure S1).

2.2 | Plant growth, crosses, and harvesting

Plants were grown in three different conditions. The soil was Debco Premium Potting Mix:special = 1:1 with 3 g L^{-1}

Osmocote. Most primary material and hybrids were grown in controlled environment cabinets with a single plant per 10-cm size pot. The light and temperature conditions of day/night were 16/8 h and 23/12 ˚C. The glasshouse conditions were less controlled, the main light source was natural light with supplementary light to provide a day length of 16 h. The temperature was 23 ˚C/12 ˚C and controlled with fans. Seeds were sown in August in pots in the open air in Canberra when the lowest day temperature was above 0 ˚C. The life cycle of material in the cabinet and glasshouse was about 120–150 d, and about 150 d for material grown underfield conditions.

A flower bud from a healthy plant with corolla petals reaching approximately three-quarters the length of the sepals was selected for hybrid generation. Petals were folded back to expose the anthers, which were removed with pointed forceps. The presence of bright yellow pollen on the forceps was checked with a magnifying glass each time when removing the anthers. After anthers had been removed, the flower was pollinated with freshly dehisced anthers from flowers of the pollen donor plants. The folded petals were returned to their original position to cover the stigma. A pollinated flower was tagged on the pedicel.

When all pods were dry, plants were harvested as single plants. Some material with an early shattering trait was harvested as soon as the pods turned brown; watering was continued and seed harvested several times.

2.3 | Germination test

Seeds of parents and hybrids were surface sterilized with 20% bleach for 10 min, washed five times with sterile water, sown evenly on germination medium (water + 8% agar (w/v), pH 7.0) in the dark, 23 ˚C. The seeds were checked from 20 h after sowing. Germination was recorded at 2 hourly intervals and when the radicle had visibly emerged from the seed coat.

2.4 | Data collection

Plant growth was recorded over the period from sowing to harvesting. The measured traits were the date of seedling emergence, time of first leaf, time of first flower, and time of last flower; height at 4 wk after sowing and harvesting, height of the lowest and upper most pods; branch number, peduncle number, pod number per peduncle and per plant, seed number per pod and per plant at harvesting, biomass at 80 d after sowing and harvesting. Seed weight per plant and 100 seed weight together with seed size were measured with imageJ; cotyledon color was verified by removing a piece of the seed coat.

2.5 | Hybrid mimic selection

Hybrid Mimic selection was conducted as previously described for Arabidopsis (Wang et al., 2015). The selection criterion was based on seed weight per plant. Hybrid combinations with outstanding better parent heterosis (BPH) were selected for further study. From F2 and subsequent generations, two to three lines were grown with seeds from the highest-yielding plants. Normally, there were more than 50 plants in each F2 line, with more than 15 plants per line in the F3 generation. Parents and hybrids, if enough seeds were obtained, were grown as controls in each generation in the same environmental conditions. Jumbo2 was grown alongside the other lines as a reference standard. As the red lentil comprises the majority of the Australian lentil industry, our selection was not always based on the highest-yielding plant, but on the highest-yielding red lentil. Selection of F1-like plants was made in the cabinet or glasshouse conditions until the F4 generation. The F5 and subsequent generations were grown in a large field trial and selected based on yield per single plot.

3 | RESULTS

3.1 | Hybrid vigor in lentil crosses

To test for hybrid vigor in lentil we made hybrids between red cotyledon lentil cultivars, between red and green cotyledon lentils, and between *L. culinaris* commercial cultivars and *L. orientalis* accessions (Supplemental Figure S1). Growth pattern, flowering time, and yield were scored. Hybrid vigor of >20% in seed weight per plant (SW) (14 out of 48 hybrids) and seed number per plant (SN) (24 out of 48 hybrids) relative to the better parent was found in a high proportion of hybrids.

In 12 hybrids between four Australian cultivated *L. culinaris* cultivars, six were hybrids between red cotyledon lentils, of which two showed BPH in SN (0.2 and 7.6%), three showed BPH in SW (8.7, 34.8, and 46.4%) (Supplemental Table S1). Six of the hybrids were between green and red parents; the combination of Greenfield crossed with Jumbo2 (J×G and G×J) showed BPH in SN (34.4 and 45%) and SW (26.4 and 33.4%) when grown under controlled cabinet conditions (Supplemental Table S2). In glasshouse conditions, the hybrid, Greenfield × Jumbo2, showed 15.1% BPH, with Jumbo2 being the better parent (Supplemental Figure S2).

3.2 | Hybrid vigor in Jumbo2 and Greenfield hybrids

Greenfield × Jumbo2 hybrids were not significantly different from the parents in early vegetative growth. No BPH in plant

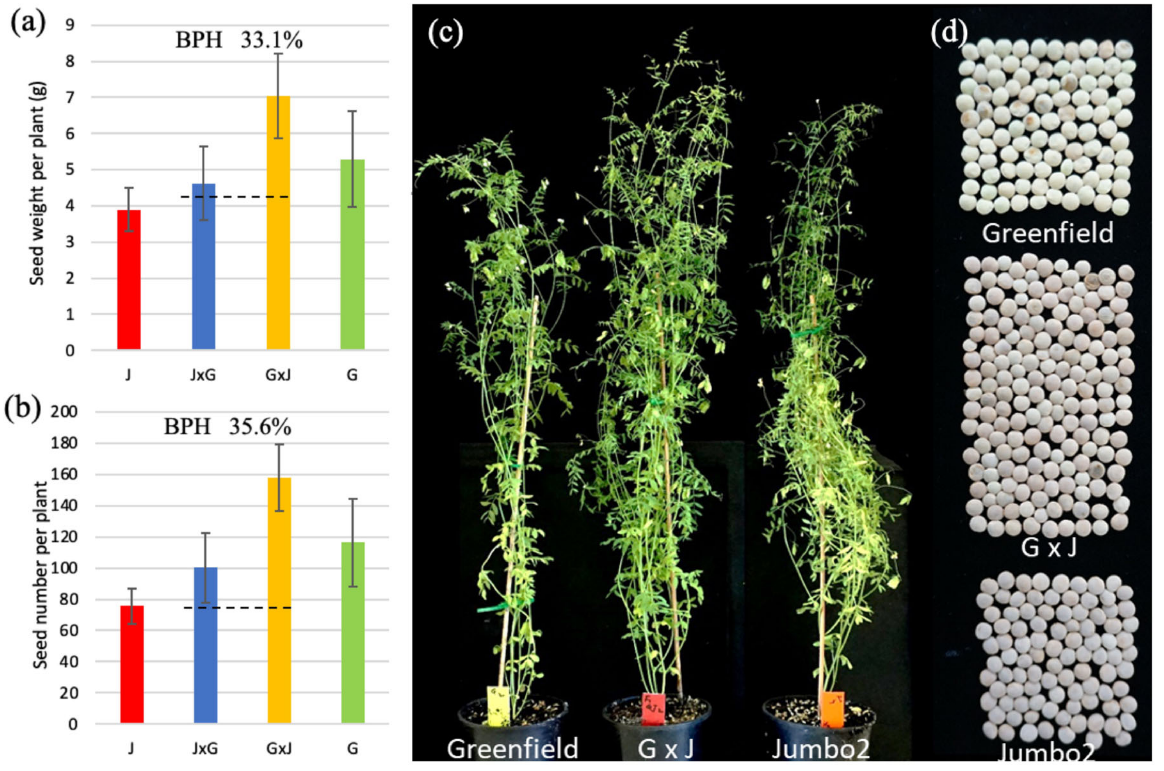

FIGURE 1 Hybrids between Australian red and green commercial cultivars, Jumbo2 (J) and Greenfield (G). (a) Seed yield weight per plant. (b) seed number per plant. J, Jumbo2, $n = 18$; G, Greenfield, $n = 15$; J×G, $n = 9$; G×J, $n = 8$. Better parent heterosis (BPH) values were calculated on seed weight and number per plant. (c) phenotype of plants in the late flowering stage. (d) Seeds from typical individual plant of different material. Dashed line indicates middle parent value (MPV)

height at 28 dps was observed in either reciprocal hybrid. BPH was found in G×J in seed yield, in both seed weight (33.1%) and seed number (35.6%) per plant (Figure 1a and 1b). Heterosis was also observed in the J×G reciprocal hybrid at the MPH level, but not at the BPH level. There is an obvious heterotic phenotype in the late stage of plant growth in G×J in both biomass and seed yield (Figure 1c and 1d).

We compared inflorescence structure, branch number, and scored flowering time and flowering duration between G×J and J×G hybrids and parents (Supplemental Figure S3). The number of pods per peduncle varied from one to three in different crosses but was not a key factor in producing high yields in the hybrid (Supplemental Figure S3a). Similar results were found for seed number per pod (Supplemental Figure S3b). Seed weight was not correlated with branch number (Supplemental Figure S3c). Although pod and seed number per peduncle varied, there was a high correlation between seed weight and peduncle number (Supplemental Figure S3d).

Greenfield flowers about 15 d later than Jumbo2. Both reciprocal hybrids flower earlier than the MPV and about 5 d later than Jumbo2 (Supplemental Figure S3e). Jumbo2 flowers early but also senesces early. The duration of flowering of Jumbo2 was approximately 20 d and the flowering duration of Greenfield and of the hybrid J×G was about 25 d. G×J showed the longest duration of flowering, about 35 d. Flowering duration is correlated with peduncle number and seed yield (Supplemental Figure S3f).

There was a high correlation between plant height and seed yield and an even higher correlation between pod stem length and seed yield (Supplemental Figure S3g and S3h). Pod stem length is the stem length from the lowest pod to the uppermost pod and is the region where productive flowers are produced.

3.3 | Heterosis was found in hybrids between green lentil accessions and Jumbo2

To increase genetic variation, we used accessions from the Australian Grains Genebank (AGG) (Supplemental Figure S1). These unrelated accessions differed in cotyledon color, early vegetative growth, flowering time, and seed size (Supplemental Table S3). Hybrids between three out of five accessions and Jumbo2 showed BPH in seed yield and outyielded hybrids crossed with Greenfield (except 73453×G, which was at the same level as 73453 × Jumbo2) (Supplemental Figure S4a). Five selected hybrids flowered later than

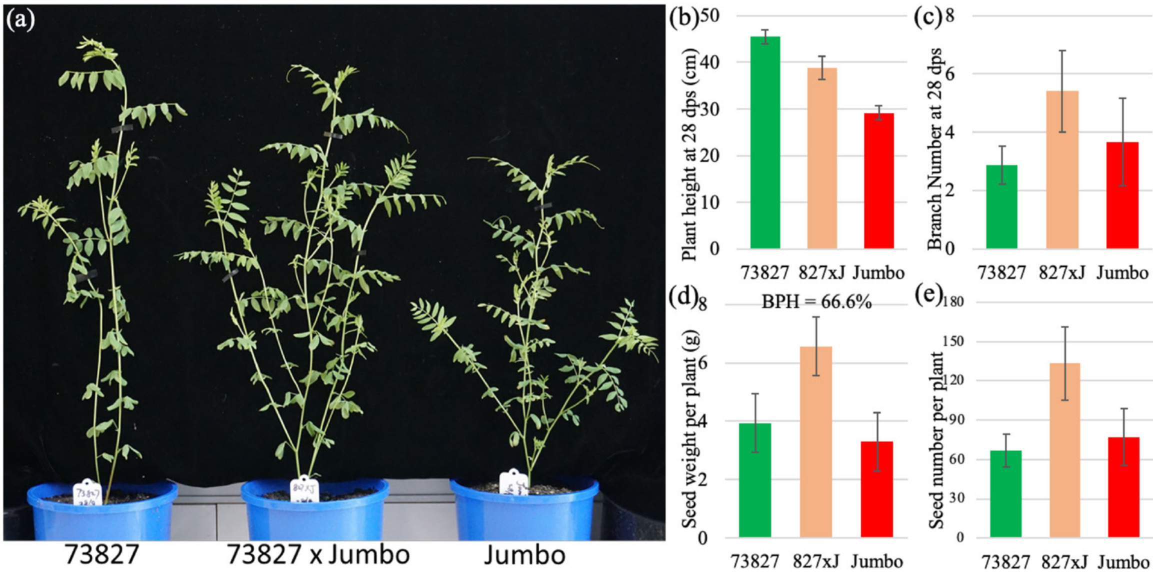

FIGURE 2 Vegetative growth and seed yield analysis of hybrid between red Australian commercial variety, Jumbo2 (Jumbo), and one Australian Grains Genebank (AGG) green lentil collection, AGG73827 (827). (a) Phenotype of parents and hybrid at 28 days post sowing (dps). (b–c) Plant height and branch number at 28 dps. (d–e) Seed yield was represented by seed weight and seed number. Better parent heterosis (BPH) was calculated based on seed weight per plant ($n = 15$)

Jumbo2 or Greenfield. Hybrids from crosses with Jumbo2 flowered earlier than the MPV, but hybrids from crosses with Greenfield flowered later than the MPV except when crosses were with line 70373 (Supplemental Figure S4b). Some of the hybrids showed greater biomass than either parent (see the 453×J from AGG73453) in the Jumbo2 hybrid (Supplemental Figure S4c).

Our results indicated seed yield heterosis occurs in many of the crosses between red and green lines (Supplemental Figure S4). Early flowering red paternal and late flowering green maternal parents appears to be a good combination for hybrid generation. To test if flowering time is a key factor, we tested another two combinations, AGG73827 × Jumbo2 and AGG70003 × Jumbo2. AGG73827 had been selected from the hybrid of AGG70003 and Precoz (Supplemental Figure S1). Both are large-seeded green advanced cultivars with similar seed size but are significantly different in flowering time (Supplemental Table S3). In glasshouse conditions, the flowering time for AGG73827 is about 32 d which is close to Jumbo2 (25 d). AGG70003 flowers at about 54 d. In the controlled cabinet conditions, there is no difference in flowering time between AGG73827 and Jumbo2, but AGG70003 flowers late. The hybrid of AGG73827 × Jumbo2 showed middle parent heterosis (MPH) in plant height and BPH in branch number at 28 dps, BPH in seed weight (66.6%) and seed number (Figure 2). At harvest, AGG70003 × Jumbo2 hybrids showed more than 60% BPH in seed yield in reciprocal hybrids (Supplemental Figure S5c).

Most green cotyledon material had medium or large-sized seed, larger than Jumbo2. Seed size is not critical for obtaining high performing hybrids. When crossed with Jumbo2, more than 20% MPH was observed in most of the hybrids. This caused us to ask if only material larger than Jumbo2 generates yield heterosis when crossed with Jumbo2. The hybrid AGG73416 × Jumbo2, where AGG73416 is a small green seed accession (about 90% of Jumbo2 in seed size), produces seed yield heterosis (Supplemental Figure S4a).

Repeated experiments under field conditions with AGG73827 × Jumbo2, AGG73416 × Jumbo2, AGG70003 × Jumbo2 and Pelagonia × Jumbo2, confirmed that flowering time and seed size might be important but not critical for seed yield heterosis in hybrids between red and green lentils (Supplemental Figure S5a and S5b). Reciprocal hybrids of AGG70003 × Jumbo2 and Pelagonia × Jumbo2 performed similarly in total seed weight heterosis (Supplemental Figure S5c and S5d).

3.4 | Hybrids showed earlier germination and more biomass

We tested if the high yielding lentil hybrids had early seed germination as was seen in hybrids of Arabidopsis and rice. In two outstanding hybrids, Greenfield × Jumbo2 (G×J) and AGG73827 × Jumbo2 (73827×J), germination begins from 24 h after sowing and reaches 100% germination of the seeds in a sample within 48 h after sowing (Figure 3). G×J hybrids showed earlier germination than parents from 24 to 34 h after sowing, no difference was observed between G×J and Jumbo2 from 34 h after sowing. Greenfield showed a delayed

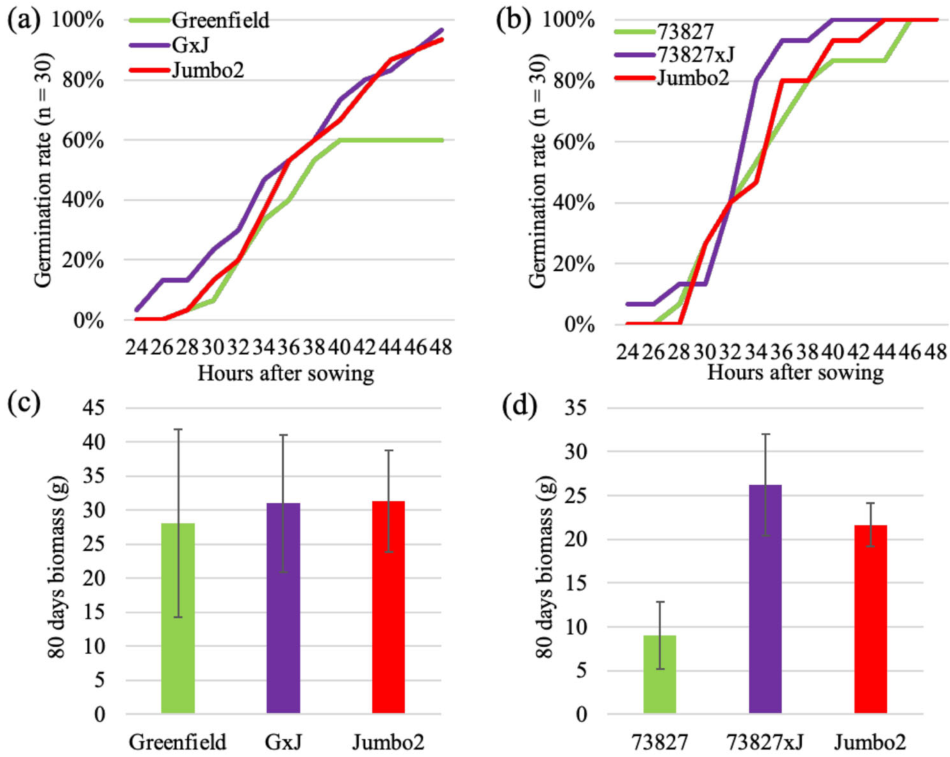

FIGURE 3 Germination and later vegetative biomass assays of (a, c) Greenfield × Jumbo2 and (b, d) 73827 × Jumbo2. ($n = 30$)

germination and a lower germination rate (Figure 3a). In 73827×J, hybrids began germination earlier than both parents (Figure 3b, Supplemental Figure S6).

Hybrids of G×J and 73827×J showed MPH and BPH on biomass at 80 d after sowing and at harvesting, respectively (Figure 3c and 3d, Supplemental Figure S6). Outstanding lentil hybrids that have substantial hybrid vigor in seed yield, also have growth vigor at both the germination stage and late vegetative growth stages.

3.5 | Heterosis from intersubspecies crossing

To examine heterosis in hybrids between cultivated and wild accessions, three *L. orientalis* accessions, AGG70956, AGG70964, and AGG70966, were crossed with the commercial cultivars, Jumbo2 and Greenfield. There was no BPH in seed weight per plant in any of the hybrids (Figure 4), but the yields of the hybrids J×956 and G×956 were comparable to the better parents Jumbo2 and Greenfield, respectively. On the other hand, all the hybrids had BPH in seed number, with J×956 and G×956 having 86 and 71% increase in BPH (Figure 4a). BPH for plant height at the harvesting stage was observed for G×956. BPH for pod stem length ranged from 10% (J×964) to >50% (J×956, 53.6%; G×956, 50.6%) (Figure 4b) and pod stem length was correlated with seed number (Figure 4a and 4b). The higher seed number in the hybrids could be due to longer flowering duration with additional flowering nodes (Figure 4c).

3.6 | A promising step towards hybrid mimic selection in lentil

Based on the findings of seed yield hybrid vigor in lentil and on Hybrid Mimic selection in Arabidopsis (Wang et al., 2015) and rice (He et al., 2021), we carried out Hybrid Mimic selection in our outstanding lentil hybrids. High-yielding plants were selected from the F1 generation, with only red seeds being selected in the next generation (Supplemental Figure S7a).

The Greenfield × Jumbo2 combination, in a field trial with parents and F1–F4 generation plants grown together, examined Hybrid Mimic selection and seed yield. The results indicated the controlled environment heterosis of G×J was repeatable in the field environment. Positive selection on seed yield by generation and high yield F5 plants in a subsequent experiment were observed (Figure 5a). To make data from different experiments comparable, we normalized the seed yield of each line in the experiment to Jumbo2 which is the paternal parent and was grown alongside the other lines in each experiment. Yield per plant increased from the F2 to the F5 generation (Figure 5b). The level of variation in yield in the F3 population was reduced in the F4 population (Figure 5a).

(a)

Material	Seed number per plant (SN)	Better parent heterosis of SN	Seed weight per plant (SW, g)	Better parent heterosis of SW
Jumbo2 (J, n = 18)	75.8 ± 11.2		3.85 ± 0.59	
Greenfield (G, n = 15)	116.3 ± 28		5.22 ± 1.31	
70964 (964, n = 6)	76.8 ± 18.5		1.17 ± 0.44	
70956 (956, n = 6)	NA		NA	
70966 (966, n = 5)	48.6 ± 21.3		0.50 ± 0.30	
Jx956 (n = 9)	141 ± 29.2	85.9%	3.65 ± 0.93	-5.2%
Jx964 (n = 9)	93.6 ± 13.6	21.8%	2.53 ± 0.28	-34.3%
Jx966 (n = 9)	95.9 ± 30.3	26.4%	2.92 ± 1.02	-24.2%
Gx956 (n = 9)	199.1 ± 82	71.2%	4.85 ± 2.37	-7.0%
Gx964 (n = 9)	132.4 ± 25.3	13.8%	3.55 ± 0.87	-32.0%
Gx966 (n = 9)	160.1 ± 54.4	37.6%	4.56 ± 1.63	-12.5%

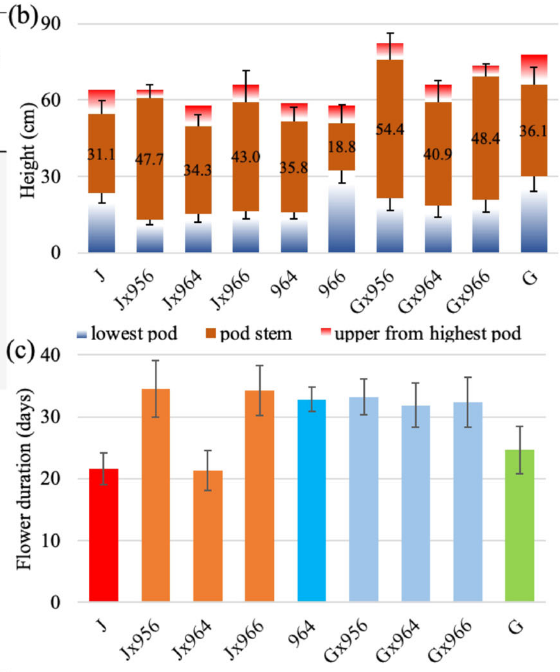

FIGURE 4 Hybrid vigor from intersubspecies hybrids. (a) Seed number and yield analysis of intersubspecies hybrids. (b) plant height (whole column) and pod stem (orange part of column) length in different parents and hybrids. (c) All hybrids except J×964 showed longer flowering period than the commercial parent

For the AGG73827 × Jumbo2 combination, in a field trial with parents, F1 and F2 generation plants were grown together. F3 and F4 generations were grown in separated subsequent experiments. The results confirmed the high BPH of this hybrid in seed yield. High yield lines were also obtained from F3 and F4 lines (Figure 5c). These data were compared by normalizing all seed yields to that of Jumbo2. Several F3 and F4 lines showed 50% higher yield than Jumbo2; these lines do not equal the outstanding F1, which is 100% higher than Jumbo2, but are much better than the F2 generation (Figure 5d).

Hybrid Mimic selection was used in the AGG73416 × Jumbo2 hybrid combination (Supplemental Figure S7b and S7c). We grew parents, F1, F2, and F3 together in one set of experiments; in the F4 generation the Jumbo2 parent was grown as a control. There was an increase of yield and decrease of variation from F2 to F3. An increased yield of F4 relative to Jumbo2 was found in two independent experiments (Supplemental Figure S7b) Normalization of all yield data to the red parent, Jumbo2, showed a trend of yield increase from F2 to F4 (Supplemental Figure S7c).

In Hybrid Mimic selection in the intersubspecies combination, J×966, data from the F1 to F4 generations indicated a high seed number per plant (Supplemental Figure S8). The seed yield per plant of the Hybrid Mimic was close to the F1 level and increased single seed weight each generation to a level higher than the F1 hybrid.

4 | DISCUSSION

4.1 | Heterosis in lentil

There are only a few studies on hybrid vigor in grain legumes, and even fewer in lentil. Traditional breeding methods have had limited potential for increasing yield. Crosses in a number of the pulses or grain legumes have shown that where hybrids have been generated by hand production of male sterility, high levels of hybrid vigor occur; this is the case in lentils where crosses between commercial cultivars and crosses involving accessions from worldwide collections produce Hybrid Mimics with yields as high as 50% greater than the commercial cultivars. As Hybrid Mimic lines are stable and overcome the restriction of the hybrid advantage to the F1, our studies have raised the possibility of using Hybrid Mimics to capture the significant yield advantage seen in hybrids. This increased yield is much greater than with conventional breeding. Hybrid Mimic selection begins with the F1 generation with high F1 vigor as the primary selection criterion.

We tested hybrids from a number of combinations, including cultivars from the same traditional breeding program and unrelated accessions from the global collections. We found 46.4% BPH in seed yield in a hybrid between the commercial lines Jumbo2 and Nugget. There was more than 30% BPH in seed yield in a hybrid of Jumbo2 and Greenfield. Nine different cultivated accessions from AGG's global collection were

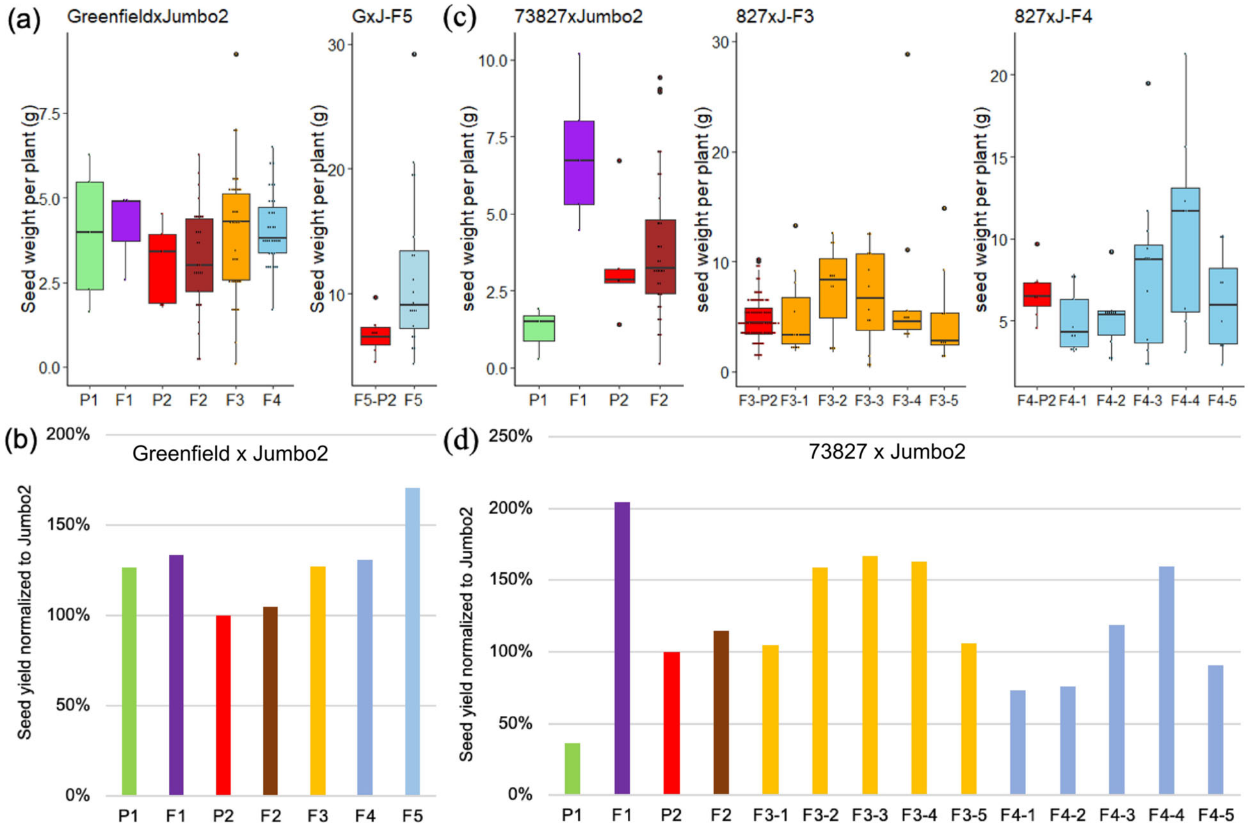

FIGURE 5 Hybrid Mimics selection in G×J and 73827×J. (a, c) Boxplot of seed weight per plant in G×J and 73827×J Hybrid Mimic selection. For G×J, two parents and F1–F4 generation plants were grown in one experiment, while the F5 generation plants were grown in a subsequent experiment along with Jumbo2 (P2). For 73827×J, two parents, F1 and F2 were grown in one experiment, while F3 and F4 generation plants were grown in subsequent experiments along with P2. (b, d) The seed weight per plant of all material was normalized to the red parent, Jumbo2, that was grown in each experiment

crossed with the two representative Australian commercial cultivars, Jumbo2 and Greenfield (Supplemental Tables S1 and S2). Six of the hybrids involving Jumbo2 had BPH of 10–122% in seed yield, but only one hybrid (73453×G) showed BPH when crossed with Greenfield (Supplemental Figure S4). The vegetative growth analysis of 73827×J showed BPH for branch number at 28 dps (Figure 2). Seed yield heterosis was reproducible in different growing environments, although the heterosis values differed. Hybrids showing high heterosis in the controlled cabinet environment also showed heterosis in both the less-controlled glasshouse and field environments (He et al., 2021; Zhu et al., 2017), hybrid vigor occurred at all stages of the life cycle and was observed as early as seed germination and in late vegetative growth (Figure 3, Supplemental Figure S6).

Six hybrids with three *L. orientalis* accessions crossed to the two Australian commercial cultivars, Jumbo2 and Greenfield had MPH in plant height at 28 dps and in seed weight per plant. The BPH of seed number was found in all the six hybrids (Figure 4). The data indicate there is heterosis in many hybrids in vegetative growth and/or seed yield. Both traits could be important in lentil breeding programs.

4.2 | Traits important for yield heterosis

We asked if there is any particular trait which could be associated with seed yield heterosis in hybrids between commercially grown lentils. Based on the analysis from the hybrids of Greenfield × Jumbo2, we found pod number per peduncle and seed number per pod were not correlated with seed yield. The major peduncles of parents and hybrids have two pods, but there is not a significant difference in pod number in the lines. Both parents and hybrids have similar seed numbers per pod, with a single seed per pod being most frequent (89.4% (G×J) to 92.4% (Jumbo2)) (Supplemental Figure S3b). The correlation coefficients (R^2) between seed yield and branch number and flowering time are .134, and .098, respectively (Supplemental Figure S3c and S3d). There is a correlation (R^2 = .391) between the length of flowering time and peduncle number (Supplemental Figure S3f). Seed weight per plant is highly correlated with peduncle number (R^2 = .746) (Supplemental Figure S3e). A hybrid with high BPH in seed yield is likely to show MPH or even BPH in plant height at 28 dps (Figures 1 and 2). Plant height and pod stem length may be useful traits to predict seed yield heterosis in hybrids growing in controlled cabinet or glasshouse conditions (Figure 4 and Supplemental Figure S3).

In some hybrids from accessions crossed with Jumbo2 (Supplemental Figure S4), flowering time, seed size, and direction of crosses, appear not to be critical for a high level of hybrid vigor; a number of female parents with different phenotypes in these traits produce heterosis when crossed with Jumbo2. Cotyledon color may be more important. We found there is a greater chance of obtaining higher yielding hybrids when the accessions (green) are crossed with Jumbo2 (red) than when crossed with Greenfield (green) (Supplemental Figure S4). Like the situation in maize where hybrids are generated from two different heterotic pools, red and green seeded lentils may be able to be used as separate pools which are more likely to generate heterotic progeny than crosses within pools.

In hybrids of accession 70003 × Jumbo2 and Pelagonia × Jumbo2 there were similar levels of BPH in the reciprocal hybrids (Supplemental Figure S5c and S5d), which differed from the Australian combinations (Greenfield × Jumbo2), where one reciprocal hybrid outperformed the other.

4.3 | Potential applications in lentil breeding

Our results show heterosis can be generated in a wide variety of lentil hybrids and could substantially boost yields. Hybrid Mimic selections in both Arabidopsis and rice (He et al., 2021; Wang et al., 2015) suggested high yields can be expected starting from a few hand-generated hybrids. Hybrid Mimic lines could be used to capture high yield traits into pure breeding stable lines. In lentil, our data from the F1 to F5 generations of the commercial hybrids, G×J (Figure 5a and 5b), the hybrids AGG73827 × Jumbo2 (Figure 5c and 5d), AGG73416 × Jumbo2 (Supplemental Figure S7b and S7c), and an intersubspecies combination, J×966 (Supplemental Figure S8), all indicated the possibility of Hybrid Mimic selection to increase seed yield and reduce variation within the mimic lines. Relatively wide variability in seed yield was observed among the heterozygous F1 plants, selected Hybrid Mimics and even homozygous parent plants (Figure 5). This could be because seed yield per plant is a complex trait. As well as genetic factors, many different environmental factors, such as light, water, and temperature, influence seed yield. A trend of increased seed yield in four different independent experiments suggests a benefit of using Hybrid Mimics in lentil breeding.

Outstanding lines and plants showing yields greater than F1 plants were observed in different experiments (Figure 5), consistent with the results from Hybrid Mimic selection in Arabidopsis and rice (He et al., 2021; Wang et al., 2020).

Our results provide a situation in lentil crop improvement where the combination of hybrids and Hybrid Mimics could be used to enhance yields. The exploration and application of hybrid vigor and Hybrid Mimic lines could be of value in a wider range of crops.

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AUTHOR CONTRIBUTIONS

Jiafu Tan: Conceptualization; Data curation; Formal analysis; Writing – original draft; Writing – review & editing. Limin Wu: Data curation. Jim Peacock: Conceptualization; Formal analysis; Funding acquisition; Supervision; Writing – review & editing. Elizabeth S. Dennis: Conceptualization; Formal analysis; Funding acquisition; Supervision; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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