Seeking long-lasting resistance to black node disease in common beans: Development of interspecific populations

Buscando una resistencia duradera a la enfermedad del nudo negro en frijol común: desarrollo de poblaciones interespecíficas

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***Phaseolus vulgaris* x *Phaseolus polyanthus*: improved red bean line.**

Photo: G.A. Ligarreto-Moreno

ABSTRACT

There are no known common bean materials that are resistant to the black node disease (*Boeremia noackiana* [Allesch.] Aveskamp, Gruyter & Verkley). However, some studies have reported common bean genotypes that exhibit an intermediate reaction to this disease, but these materials lack stability in this trait. The secondary gene pool for beans (*Phaseolus polyanthus* Grenm.) has been screened since 1995 for this resistance. This population shows a varied response to this disease but lacks the characteristics of commercial grains. The study was carried out during 2017-2018 in Bogota under greenhouse conditions with interspecific crosses of commercial bean varieties (*Phaseolus vulgaris* L.) in Colombia and resistant genotypes from the ASC population of *P. polyanthus,* CIAT origin. To carry out the crosses, the hybridization technique with emasculation was followed, both in direct and reciprocal crosses. Low-efficiency viability in interspecific crosses from cytoplasmic genetic compatibility problems has been reported in different studies. However, in this study, the efficiency of the percentage of viable interspecific crosses increased significantly in the F1 populations and backcrosses, reaching 67%. Interspecific populations of Bacata × ASC 160 and Bacata × ASC 162 were formed with the seeds, which constituted the starting point for a breeding program for resistance to the black node disease in common beans using susceptible commercial cultivars.
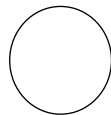
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RESUMEN

No se conocen materiales de fríjol común que sean resistentes a la enfermedad del nudo negro (*Boeremia noackiana* [Allesch.] Aveskamp, Gruyter & Verkley). Sin embargo, algunos estudios han reportado genotipos de fríjol común que exhiben una reacción intermedia a la enfermedad, pero estos materiales carecen de estabilidad para esta característica. El acervo genético secundario del fríjol (*Phaseolus polyanthus* Grenm.) ha sido evaluado desde 1995 en búsqueda de dicha resistencia. Esta población muestra una respuesta variada a la enfermedad, pero carece de las características comerciales del grano. El estudio se realizó durante 2017-2018 en Bogotá bajo condiciones de invernadero, a través de cruces interespecíficos de variedades comerciales de fríjol (*Phaseolus vulgaris* L.) en Colombia con genotipos resistentes de la población nudo negro de *P. polyanthus* de origen CIAT. Para realizar los cruces se siguió la técnica de hibridación con emasculación, tanto en cruces directos como recíprocos. La baja eficiencia en la viabilidad de cruces interespecíficos debido a aparentes problemas de compatibilidad genética citoplasmática ha sido reportada en diferentes investigaciones. Sin embargo, en este estudio la eficiencia del porcentaje de cruces interespecíficos viables aumentó significativamente en las poblaciones F1 y retrocruzamientos, llegando a 67%. Con las semillas obtenidas se conformaron poblaciones interespecíficas de Bacatá × ASC 160 y Bacatá × ASC 162, que constituyen el punto de partida de un programa de mejoramiento para resistencia a la enfermedad de nudo negro en fríjol común a partir de cultivares comerciales susceptibles.

INTRODUCTION

The black node disease is a severe fungal alteration that affects beans in regions with cold to moderate temperatures (15-25°C), continuous rainfall, and high relative humidity (80-100%). In the Americas, the Black node disease is commonly found in cold climate regions at more than 1,500 m a.s.l. Infestation can be very severe, causing 100% yield loss in bush beans in Ecuador, Colombia and Peru, among other countries in the Andean region of South America, and is also significant in high elevation areas of Guatemala in Central America (Garzón *et al.*, 2011; Miranda *et al.*, 2021).

Sustainable production strategies in current agri-food systems are important for reducing unfavorable impacts on the environment and generating products that alleviate malnutrition for millions of people (Powers and Thavarajah, 2019). The common bean is of interest when improving food systems because of its great diversity and use of genetic recombination that transfers helpful genes for resistance to biotic and abiotic factors (Montejo *et al.*, 2022). Recombination is achieved from artificial crosses between genotypes of interest, either at the intraspecific or interspecific level (Singh *et al.*, 1991; Montejo *et al.*, 2022). This technique is widely used in bean breeding programs

in search of resistant materials and increased dry grain yield (Barrios *et al.*, 2011). However, research on this topic has been limited by the lack of continuity and the scarce publication of studies on interspecific breeding in beans. In addition, there are no reports on the mechanisms of inheritance of resistance to some abiotic factors and limiting diseases for bean production, as is the case for black node (*Boeremia noackiana* [Allesch.] Aveskamp, Gruyter & Verkley).

According to Beebe and Corrales (1991), intraspecific crosses do not usually show problems as opposed to interspecific crosses that frequently display incompatibility between materials. Intraspecific crosses are used when it is necessary to introgress genes of interest and are widely applied to transfer genes with resistance to pathogens (Silue *et al.*, 2014).

Hanson *et al.* (1993) reported that there are no known *Phaseolus vulgaris* L. materials with resistance to the black node disease caused by the pathogen *Boeremia diversispora* (Bubák) Aveskamp, Gruyter & Verkley. However, several studies have reported genotypes with an intermediate reaction (Garzón *et al.*, 2011). Thus, the ASC population from the *Phaseolus polyanthus* Grenm. resistant genotype G35575 developed

by the International Center for Tropical Agriculture (CIAT) was explored. This species has a close evolutionary relationship with *P. vulgaris* (Chávez, 2017).

Segregating populations of these interspecific crosses are unstable, especially given their reversal to *P. vulgaris* when this species is used as the female parent. However, reciprocal crosses using *P. polyanthus* as a female parent attenuate this reversal to *P. vulgaris*. Thus, the recombination frequency for tolerance to *B. noackiana* that is typical of *P. polyanthus* is increased, while maintaining the fertility and grain quality of *P. vulgaris* (Camarena and Baudoin, 1987).

The objective of this study was to develop populations of filial generations with possible genetic resistance to the black node disease (*B. noackiana*) in susceptible common bean cultivars with commercial grain characteristics using the introgression of desirable *P. polyanthus* genes.

MATERIALS AND METHODS

This study was carried out under greenhouse conditions in the facilities of the Faculty of Agricultural Sciences at the Universidad Nacional de Colombia, Bogotá campus during 2017-2018. Permanent cultivation was established under tropical climate conditions, at an altitude of 2,650 m a.s.l., with an average annual temperature of 18.6°C, maximum and minimum temperatures of 40 and 6 °C, respectively, and average relative humidity of 70%.

The plant materials were ASC 160, ASC 162, and NCB 226 provided by CIAT, within the framework of the project "Analysis of the distribution and genetic

and pathogenic diversity of ascochyta (*Boeremia* spp.) in beans as a tool for the development of improved cultivars and small agriculture in Colombia". The first two materials were obtained from the progeny of the resistant parent G35575 (*P. polyanthus*) and CAL 96 (*P. vulgaris*), with 16 successive generations of individual and mass selections, from CIAT's genebank. These materials show high resistance with disease reaction values of 1 for leaves and 2 for pods according to the CIAT's scale for the pathogen *Boeremia* spp. (Schoonhoven and Pastor-Corrales, 1987). The materials were selected for their resistance and genetic closeness to *P. vulgaris*. *Phaseolus polyanthus* is believed to be a natural interspecific cross between cultivated forms of *P. vulgaris* and *Phaseolus coccineus,* which could facilitate the development of populations of filial generations (Silue *et al*., 2014). The material NCB 226 (*P. vulgaris*) was used as the susceptible control.

P. vulgaris commercial cultivars requiring resistance to this pathogen were selected for their traits of grain size, color, shape, and consumer acceptance. The cultivars were: Sutagao, an improved climbing cultivar with red seeds, and Bacata, an improved shrubby cultivar with red seeds. The regional varieties Simijaca and Cabrera, both with red seeds, were used as controls (Tab. 1).

Two sowing systems were used: first, seeds were sown directly in the soil; six 20 m long rows were used at distances of 1.1 m between rows and 0.2 m between plants, for an area of 132 m^2. The parental plant was sown at 1.5 linear m for shrubby beans and 2 linear m for climbing beans. The sowing was carried out in complete rows, staggered for all genotypes. The second sowing system was in 3 kg pots with a mixture of soil and rice husk plus 20 g of the

Table 1. Bean cultivars used for interspecific crosses.

Cultivar	Commercial type	Growth habit	Reaction to black node disease°	Days to flowering	100-grain weight (g)
Bacata*	Red kidney	Shrubby	Susceptible	60-65	64.15
Simijaca*	Red round shape	Climbing	Unknown	114	117.03
Sutagao*	Red round shape	Climbing	Unknown	90	69.16
Cabrera*	Red round shape	Climbing	Susceptible	109	114.94
NBC 226	Non-commercial***	Shrubby	Susceptible°°	30**	34.30
ASC 160		Shrubby	Resistant°°	35**	24.08
ASC 162		Shrubby	Resistant°°	35-45**	27.15

Source: * Bean breeding program of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogota. ** Under greenhouse conditions in Bogota - Colombia. *** Considering the demand in Colombian markets. ° Reaction to the disease: 1-3 resistant, 4-6 intermediate reaction, 7-9 susceptible. °° CIAT sources (2017).

commercial product 15-15-15 (NPK) for edaphic fertilization, as well as foliar fertilization with amino acids and microelements at 30, 40, and 60 days after sowing (DAS). Additionally, adequate management was carried out to control typical insect pests in common beans, such as whitefly (*Trialeurodes vaporariorum* Westw.), mites (*Tetranychus* sp.), thrips (*Frankliniella* sp.), and cutworms (*Agrotis ipsilon* Hufn.). Fungicides were also applied to manage powdery mildew (*Erysiphe polygoni*). The two sowing systems were complementary, for better use of the availability of flowers for crosses since the genotypes had very dissimilar life cycles up to flowering (Tab. 1). Therefore, the sowing methods were not quantified.

The hybridization technique with emasculation was used for the crosses. This method ensures that the resulting seed is the progeny of the cross between two different parents. This procedure was suggested by Pimentel *et al*. (2021), which consisted of taking

Table 2.   Direct crosses and variables related to the efficiency of interspecific crosses.

Female parent *P. vulgaris*		Male parent *P. polyanthus*	Total crosses	Viable crosses	Efficiency (%)	Seeds per pod	Number of seeds*
Bacata	×	ASC160	609	70	12	0.60	45
Bacata	×	ASC162	693	64	9	0.61	48
Cabrera	×	ASC160	2,289	210	9	0.37	70
Cabrera	×	ASC162	1,368	55	4	0.16	9
NBC226	×	ASC160	1,332	102	8	0.65	25
NBC226	×	ASC162	1,177	108	9	1.54	27
Simijaca	×	ASC160	2,152	152	7	0.24	30
Simijaca	×	ASC162	1,330	85	6	0.25	21
Sutagao	×	ASC160	1,170	128	11	0.20	26
Sutagao	×	ASC162	935	40	4	0.05	2
Total			13,055	1014			303
Mean					8	0.47	30.3

*Good quality seed, seeds with problems were discarded when counting seeds.

Table 3.   Reciprocal crosses and variables related to the efficiency of interspecific crosses.

Female parent *P. polyanthus*		Male parent *P. vulgaris*	Total crosses	Viable crosses	Efficiency (%)	Seeds per pod	Number of seeds*
ASC160	×	Bacata	315	18	6	0.80	8
ASC162	×	Bacata	168	6	4	1.00	3
ASC160	×	Cabrera	618	36	6	0.14	3
ASC162	×	Cabrera	378	12	3	1.00	3
ASC160	×	NBC226	1,113	21	2	0.00	0
ASC162	×	NBC226	546	24	4	2.00	12
ASC160	×	Simijaca	924	96	10	0.71	10
ASC162	×	Simijaca	441	18	4	2.00	12
ASC160	×	Sutagao	224	18	8	0.75	10
ASC162	×	Sutagao	126	0	0	0.00	0
Total			4,853	249			61
Mean					5	0.84	6.1

*Good quality seed, seeds with problems were discarded when counting seeds.

a flower bud (representing the mother plant) and opening the petals with fine-tipped tweezers; then, the standard was opened by the suture, accessing the two keel petals to remove the anthers. The male parent was a completely open flower; as in the previous step, the standard and the keel were pulled in opposite directions with tweezers. The stigma that was covered with viable pollen from the mature anthers was extracted. In the final step, the stigmas were rubbed together and left intertwined on the mother's flower bud to provide more time for pollination.

Blair *et al.* (2011) and Garzón *et al.* (2011) determined the days to anthesis for some cultivars when working on resistance to *Boeremia* spp. This information, along with the growth habit of the cultivars, was considered for the sowing. Shrubby materials flower between 30 and 65 DAS, while variable materials flower on average at 95 DAS (Tab. 1).

To obtain F1 seeds, the homozygous lines ASC 160 and ASC 162 (resistant to the disease) were crossed with five cultivars, four of them of commercial grains adapted to high tropics, and the non-commercial cultivar NBC 226 (Tab. 1). The direct cross was susceptible parent 1 × resistant parent 2, and the reciprocal cross was resistant parent 1 × susceptible parent 2 (Tab. 2 and 3). A cross was viable when it produces a pod with at least one seed, the efficiency of the cross is the percentage of viable crosses out of the total number of crosses made for each pair of parents, and the number of seeds per pod results from the average number of seeds of viable crosses once deformed, fractured and very small seeds are discarded.

For the second cycle of crosses, the best parent of *P. vulgaris* was taken in the interspecific crosses because of greater efficiency in the number of viable crosses and seed quantity. The parents and F1 were sown in a staggered manner, where F2 populations were obtained with self-pollination in some hybrid seeds. Backcrosses (BC) were carried out from the crosses between F1. BC1 was the cross between F1 and its female parent, and BC2 was the cross between F1 and its male parent (Tab. 4).

No experiment design was used to obtain the bean progeny; the treatments corresponded to the families. The variables recorded per plant were: Number of total crosses for each resistant parent, number of viable crosses, date of crossing, and seed quantity. Differences between treatments were determined using t-tests (*P*<0.05) with SAS®.

RESULTS AND DISCUSSION

The broad crossing plan in this study consisted of three genotypes susceptible to *B. noackiana* and three genotypes with characteristics of commercial grain for Colombia with an unknown reaction to *B. noackiana,* which were crossed with three resistant genotypes from CIAT (Tab. 1). According to Muñoz and

Table 4. Populations of interspecific crosses between *P. vulgaris* and *P. polyanthus*: F1, F2, and backcrosses (BC) and efficiency of crosses.

Female parent	Male parent	Total crosses	Viable crosses	Efficiency (%)	Seeds per cross	Quantity of seed	Mean seed weight (g)
Bacata	ASC160	156	16	10	2.80	46	0.92
F1 (Bacata × ASC160)	ASC160	48	18	38	1.50	30	0.67
F1 (Bacata × ASC160)	Bacata	32	10	31	2.20	22	0.53
F2 (Bacata × ASC160)*		28	29		2.00	51	0.73
Mean				26	2.13		0.71
Bacata	ASC162	118	18	15	2.50	45	0.76
F1 (Bacata × ASC162)	ASC162	48	32	67	2.43	78	0.82
F1 (Bacata × ASC162)	Bacata	13	8	62	2.13	17	0.70
F2 (Bacata × ASC162)*		30	30		1.50	45	0.76
Mean				48	2.14		0.76

* Product of F1 self-pollination.

Hidalgo (1986), the use of a wide range of genotypes with different origins, seed types, and growth habits makes it possible to significantly increase interspecific hybrids. Consequently, to increase the success of crosses, cultivars adapted to the Andean zone were used, and a high number of pollinations were carried out. Toussaint *et al*. (2004) carried out studies at the Faculty of Agricultural Sciences of the University of Gembloux with the genotypes: G 21245 wild form (WLD) and NI 637 cultivated form (CV) in *P. vulgaris*, NI 1015 (CV) and G 35348 (CV) in *P. Polyanthus,* CIAT origin, and reported abnormalities observed during embryo development, which depended to a great extent on the compatibility between the crossed genotypes. This showed the difficulties caused by interspecific crosses and the slow progress when this type of cross is carried out in a breeding program.

Of all the possible direct or reciprocal crosses between the five susceptible genotypes of *P. vulgaris* and the two resistant genotypes of the ASC population, the direct crosses where *P. vulgaris* was the female parent showed the highest efficiency. On the other hand, when the resistant materials were used as the female parent, the efficiency dropped drastically, reaching values of zero. This difference was significant according to the Student's t-test ($P \leq 0.05$). Tables 2 and 3 summarize the number of crosses, as well as the variables related to the percentage of crossing efficiency.

The detachment of immature pods and the low generation and viability of seeds stood out among the factors that affected the efficiency between crosses in this study, whether direct or reciprocal. These problems coincide with those described by Gepts (1981), specifically for interspecific crosses of *P. vulgaris* with *P. coccineus* and *P. polyanthus*. However, the crosses described in tables 2 and 3 provide a viable option to prioritize crosses based on greater efficiency and seed production.

According to Hadley and Openshaw (1980), one of the possible causes of pod abscission is low embryo viability, which results in the loss of F1 hybrid zygotes or developing embryos generated by successful pollination. Ibrahim and Coyne (1975) were able to maintain a high percentage of developing pods from interspecific crosses in *Phaseolus* for 30 d with the help of hormones, as compared to pod abortion or abscission in the control treatment 15 d after pollination. Several studies have achieved normal plant growth and development, obtaining hybrid plants through *in vitro* culture of immature embryos

(Geerts *et al*., 2011). There are different options to expand the genetic base of bean cultivars through interspecific hybridization; however, because of the low costs, conventional breeding with crosses in low-resource programs may be the most viable option.

Another important factor in the formation of interspecific hybrids was the lower efficiency in reciprocal crosses, as compared to direct crosses when the ASC population was used as the female parent. This result agrees with the studies cited by Gepts (1981). Camarena and Baudoin (1987) reported that the direction of crossing results from the nucleus-cytoplasm interaction, which does not occur when the female parent is *P. vulgaris*. Gepts (1981) suggested that the combination of parents is of interest in the success rate. Thus, if the female parent is *P. vulgaris*, there is an average of 1.2 seeds per pollination; whereas, if the cross is reciprocal (i.e., *P. coccineus* is the female parent), only 0.004 seeds are obtained per pollination. This behavior was observed in this study; even when the success rate in the cross Bacata × ASC 160 had an efficiency of 12% and a mean number of seeds per pod of 0.6, its reciprocal cross had an efficiency of 6% and a mean number of seeds per pod of 0.8, which were higher than in the other tested combinations. Cultivars Bacata and Simijaca may have lower specific genetic barriers that allow greater interspecific hybridization efficiency. In addition, efficiency varies according to the source of resistance. This can be attributed mainly to the genetic differences of the parents, as suggested by Gepts (1981), and to the adaptation of the cultivars to the environmental conditions of the experiment when making the crosses (Berke, 2000).

According to Ibrahim and Coyne (1975) and Silue *et al*. (2014), the transfer of a trait from one species to another is always accompanied by a sterility effect. The hybrid is easier to obtain when *P. vulgaris* is used as the female parent, but the degree of sterility in F1 is higher than in reciprocal hybrids. Similar results were obtained in this study since the mean crossing efficiency was 8% when the female parent was *P. vulgaris;* whereas, for the reciprocal cross, the mean crossing efficiency was 5%. However, the mean number of seeds per pod was higher for the reciprocal cross with *P. polyanthus* as the female parent (Tab. 2 and 3). Manshardt and Waines (1983) stated that the gametes of the zygotes had a high proportion of *P. vulgaris* alleles from the cytoplasm of the same species, displaying a higher degree of selectivity. Chen *et al.* (1981) studied F1 lines from bean interspecific

hybrids and reported that meiosis was abnormal, possibly because of an interaction between the genome and the cytoplasm or interactions between chromosomes. In the case of F1 hybrids of *P. vulgaris* crossed with *P. coccineus*, a close relative of *P. polyanthus* (Chávez, 2017), abnormal meiosis was reported, where at least two pairs of chromosomes were differentiated by inversions. Studies on anaphase I and II pollen mother cells have indicated that inversions are located at the end of differentiated chromosomes and are responsible for up to 79% of pollen abortion in interspecific hybrids (Cheng *et al*., 1981).

For the second cycle of crosses from the five susceptible parents, cultivar Bacata was selected as one of the more efficient in both direct and reciprocal crosses with both ASC sources of resistance in the first cycle of crosses (Tab. 2 and 3). 'Bacata''s precocious life cycle facilitated the formation of populations of subsequent BC generations and F2. Table 4 shows that the two F1s, both 'Bacata' with 'ASC160' and 'Bacata' with 'ASC162', exhibited a crossing efficiency equal to or greater than 10%. When this progeny was crossed again with its parents, the crossing efficiency of the new BC was increased with values that ranged between 31 and 67%. This difference was significant according to the Student's t-test ($P \leq 0.05$). For the sources, 'ASC 162' showed the highest crossing efficiency without differences with 'ASC 160' in the mean number of seeds per pod and seed weight. The results are summarized in table 4.

In the populations generated from the parent 'Bacata' (F1, BC, and F2), the mean number of seeds obtained per cross was 2.13. This value was higher than that reported by Gepts (1981), who obtained a mean of 1.3 seeds. The better performance in the 'Bacata' populations may have been due to the fact that the ASC population was used. This population comes from a simple cross between *P. vulgaris* and *P. polyanthus,* which has been increased during several cycles and has greater adaptation than F1 populations from recent interspecific crosses. Therefore, the ASC lines may work as 'genetic bridges', being compatible with the selected 'Bacata' parent; whereas, the values reported by Gepts (1981) were the result of a simple cross between *P. vulgaris* × *P. polyanthus* parentals.

Despite the low rate of seed production per crossing, the efficiency of the crosses varied significantly between the populations of single F1 crosses, backcrosses (BC), and F2 of 'Bacata'. This behavior was similar to that observed by Gepts (1981), who indicated that

efficiency depends on the combination of the parents and that subsequent generations have a progressive restoration of the viability and fertility of the populations. In this study, the backcross (Bacata × ASC 162) × ASC 162 reached an efficiency of 67%.

The fact that the backcrosses with the sources of resistance, 'ACS 160' and 'ACS 162', have shown crossing efficiency values between 31 and 67%, a valuable contribution to broadening the useful genetic base. The ability to exploit it for all traits of agronomic value that can be transferred from the *P. polyanthus* source provides a great opportunity for breeding for resistance. According to Camarena and Baudoin (1987), early generations of *P. vulgaris* × *P. polyanthus* and *P. coccineus* crosses are characterized by a reversal to *P. vulgaris,* with a loss of 50% of the traits of the sources of resistance in each generation because of the interaction between the cytoplasm and the genome. This was avoided in the present study since the ASC sources were used as the female parent of the F1 of Bacata × ASC 160 or Bacata × ASC 162 from backcrossings. Additionally, these crosses obtained 30 and 78 seeds, respectively, which means that using backcrosses may see a rapid reversal of the traits of interest to the recurrent parent in subsequent generations. In the case of the 'ASC 162' source, the seed weight was 0.82 g, a large size. This is an important factor for grain selection processes for commercial characteristics in high Andean bean production systems.

Consequently, it was possible to obtain enough seeds to continue developing populations of advanced generations for selection processes of breeding lines with varietal resistance. These results suggested that it is possible to perform introgression of genes for resistance to the black node disease (*B. noackiana*) in common beans (*P. vulgaris*) through interspecific crosses with *P. polyanthus* using conventional procedures. To continue the studies on greater efficiency in the use of genetic variability in *P. vulgaris*, research that determines the deficiency in endosperm development and failures in the transport of nutrients from the female parent tissue to the zygote can complement the information reported by Geerts *et al*. (2002) and Nguema *et al*. (2007).

CONCLUSIONS

The genotypes (CIAT) ASC 160 and ASC 162, which are resistant to the black knot disease (*Boeremia noackiana*), showed greater genetic compatibility with

P. vulgaris when used as male parents, with 8% cross viability. The viability in the reciprocal crossings was 5%. These values are higher than those reported in previous studies on the formation of interspecific hybrids in bean breeding programs in the Andes. The shrub-type Bacata and the climbing Simijaca were more compatible with the source genotypes of resistance. Thus, the Bacata × ASC 160 and Bacata × ASC 162 populations and the Simijaca parent, when used as a male parent, can constitute the basis for an interspecific bean improvement program for varietal resistance since they produce viable seeds with grain characteristics close to commercial ones.

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