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Registration of 'UC Rio Zape' heirloom-like dry bean

Travis Parker | **Antonia Palkovic** | **E. Charles Brummer** | **Paul Gepts**

Dep. of Plant Sciences/MS1, Section of Crop & Ecosystem Sciences, Univ. of California, 1 Shields Ave., Davis, CA 95616-8780, USA

Correspondence
Travis Parker, Dep. of Plant Sciences/MS1, Section of Crop & Ecosystem Sciences, Univ. of California, 1 Shields Ave., Davis, CA 95616-8780, USA.
Email: trparker@ucdavis.edu

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Abstract

'UC Rio Zape' (Reg. no. CV-335, PI 693471) is a dry bean (*Phaseolus vulgaris* L.) developed by recurrent backcrossing between the landrace Rio Zape (recurrent parent) and 'Matterhorn' great northern bean (donor parent). UC Rio Zape is a $BC_5F_{2:7}$ cultivar that traces approximately 98% of its ancestry to Rio Zape but demonstrates resistance to *Bean common mosaic virus* due to introgression of the *I* gene. UC Rio Zape also has yields 19% higher than its heirloom parent. It also maintains culinary quality comparable to its heirloom parent. All field evaluations were conducted on certified organic land. UC Rio Zape will serve growers and consumers interested in heirloom-like bean that can be produced efficiently and economically.

1 | INTRODUCTION

'UC Rio Zape' (Reg. no. CV-335, PI 693471) is a $BC_5F_{2:7}$ dry bean (*Phaseolus vulgaris* L.) cultivar developed by recurrent backcrossing between the landrace Rio Zape (recurrent parent) and 'Matterhorn' great northern bean (donor parent, PI 604228; Kelly, Hosfield, Varner, Uebersax, & Taylor, 1999). The cultivar has the pedigree Rio Zape*6/Matterhorn. The purple and black striped pattern of UC Rio Zape is similar to that of the heirloom recurrent parent (Figure 1). Heirloom genotypes of common bean, such as Rio Zape, have high market value due to a reputation for high culinary quality and desirable seed coat patterns (Brouwer, Winkler, Atterberry, Jones, & Miles, 2016; Walters, Brick, & Ogg, 2011). Heirloom types are not well defined, but typically are described as older varieties that have existed for at least 50 yr, or which have been passed between generations (Brouwer et al., 2016, DeMuth, 1998). This is partly overlapping with the idea of landraces, which are traditional genotypes that have been selected for adaptation to a specific region. Heirloom types and landraces are not restrained to the phenotypic requirements of major commercial market classes and thus often display diversity in appearance and culinary properties. This provides a unique market opportunity for smaller scale niche growers, including those in the organic sector. Consumer-demand studies have shown that restaurant managers and consumers are, on average, willing to pay between US$10.52 and $17.60/kg for heirloom dry bean produced locally and organically (Swegarden, Sheaffer, & Michaels, 2016). Landraces with seed patterns similar to Rio Zape have been cultivated in the southwestern United States for thousands of years (Kaplan, 1956). Despite these advantages, Rio Zape is susceptible to *Bean common mosaic virus* (BCMV). Matterhorn, the donor parent and source of BCMV resistance, was developed jointly by the Michigan Agricultural Experiment

Abbreviations: BCMV, *Bean common mosaic virus*; BCMNV, *Bean common mosaic necrosis virus*.

FIGURE 1 Seed types of UC Rio Zape and Rio Zape are consistent across growing environments

Station and the USDA–ARS as a high yielding, type II indeterminate great northern bean (Kelly et al., 1999). Matterhorn carries the dominant *I* allele for BCMV resistance, which also provides hypersensitive resistance at a field level against the closely related *Bean common mosaic necrosis virus*. (BCMNV). Hypersensitive death of individual plants with *I* can occur if aphids transmit the necrotic BCMNV strains from a susceptible plant (without *I*-mediated resistance), but *I* prevents replication and spread of the virus through the field (Feng, Poplawsky, Nikolaeva, Myers, & Karasev, 2014). Bean breeding programs in North America demand BCMV resistance in all newly developed cultivars (Haley, Afanador, & Kelly, 1994; Miklas, Kelly, Beebe, & Blair, 2006), as BCMV is rapidly spread mechanically, by seed, and by aphids in susceptible genotypes (Schwartz & Pastor-Corrales, 1989). *Bean common mosaic virus* is problematic for seed production, where these routes of transmission can lead to heavy virus loads in susceptible seed stocks. Many smaller scale producers of heirloom genotypes save seed for planting in subsequent seasons, and by planting BCMV-resistant cultivars, they can avoid the need to rogue infected individuals.

Rio Zape and Matterhorn are both descended from the Middle American common bean gene pool and have type S phaseolin (Gepts, Osborn, Rashka, & Bliss, 1986; Kami et al., 1995; Parker, Palkovic, & Gepts, 2015). Matterhorn was chosen as a donor in part to eliminate the potential for hybrid inviability, which is encountered occasionally in inter-gene-pool crosses (Gepts & Bliss, 1985; Kelly, 1988, 2018; Koinange & Gepts, 1992; Hannah et al., 2007). Rio Zape, the recurrent parent of UC Rio Zape, is not protected by plant variety protection (database available at https://apps.ams.usda.gov/CMS/CropSearch.aspx) or utility patents (database available at http://patft.uspto.gov/netahtml/PTO/search-adv.htm) and is therefore suitable as a parent for recurrent backcrossing.

UC Rio Zape is intended for production in relatively dry climates, such as the southwestern United States. Currently, few specialty heirloom-like cultivars combine high culinary quality with high yields and BCMV resistance. UC Rio Zape will help fill this market demand.

2 | METHODS

The initial crosses between Rio Zape and Matterhorn were made in 2014. This was immediately followed by five further rounds of backcrossing. Individuals that were heterozygous resistant to BCMV in each generation were selected as parents, based on viral inoculations in the greenhouse and screening with a CAPS marker (Bello et al., 2014). Viral inoculations were conducted with the non-necrotic type strain (NL 1, Drijfhout, 1978) of BCMV at 7 d after planting. Inoculum was a 1:10 mixture of (a) BCMV-infected leaf tissue and (b) a 0.01 M potassium phosphate buffer solution at pH 7.4. These ingredients were combined in a mortar with approximately 5 mg of Celite and crushed using a pestle. A small amount of Celite was dusted onto the first simple leaves of emerging plants, and the inoculum was gently rubbed into the leaves with the pestle. Symptoms were evident in susceptible plants within 2 wk and were very strong about a month after inoculations. Plants that were resistant based on inoculations and CAPS marker screenings were used as parents for further backcrossing. The BC_5F_1 progeny were self-pollinated, and homozygous resistant individuals were identified using the codominant CAPS marker. UC Rio Zape is descended from one homozygous resistant backcrossed individual that was bulk harvested in all subsequent generations. UC Rio Zape and its heirloom parent were never grown in adjacent plots during on-farm trials. Instead, at least one (and usually several) plots of phenotypically different cultivars were planted between them. This greatly reduces the chance of seed mixture during harvest and also reduces the chance of cross-pollination between the two. Breeder seed was grown in a single quarter-acre block of a conventionally managed field free of BCMV-susceptible plants and with low pollinator abundance. To further ensure that no major seed mixture occurred during trialing, 15 plants were grown from breeder seed and inoculated with BCMV. None of these plants developed BCMV symptoms, whereas the susceptible control 'Zuni Gold' became infected.

The 2017–2019 plantings used randomized complete block designs. UC Rio Zape and Rio Zape were planted in each of four blocks at one location in 2017. These plots were 2.3 m^2 in area and included 30 seeds planted into 3 m of row space. In 2018 and 2019, each of the three field sites consisted of three plots of each cultivar. All plots were 9.3 m^2 in area and included 120 planted seeds in 12.2 m of row space. Each plot was 6.1 m in length, 1.5 m in width, and included two rows spaced at 0.75 m. The trials were conducted on certified organic farms, and Guard-N N_2–fixing bacteria were added to seeds before planting to ensure consistent nodulation. Collaborating farmers were all experienced with producing common bean and were asked to prepare fields as they would normally for the crop. This typically consisted of a winter cover crop and the use of compost in spring before planting. All trials were irrigated by surface drip, buried drip, or furrow irrigation. The yield of each type was compared by paired *t*-test of block data for UC Rio Zape and its heirloom parent.

The culinary properties of UC Rio Zape and Rio Zape were assessed to ensure that no reduction in flavor or cooked visual appearance was associated with its development. Flavor and cooked visual appearance desirability were each evaluated on a 1–5 scale (1 = inferior, 5 = superior). Participants were not asked to evaluate desirability on any predetermined metrics, nor were they given any training on what they "should" want in a cultivar, as these could serve as a form of bias that would not ordinarily be found in the target market. All cooked samples were given three-digit numeric descriptors and a randomized order to ensure objectivity in the evaluation. The heirloom and improved types were also cooked at the same time in identical vessels, then kept at a consistent temperature using a water bath. The taste tests were conducted at a single event. Thirty-three evaluators responded regarding the flavor of both types, while 30 responded about the cooked visual appearance of both. A paired *t*-test was used to compare the performance of each type.

3 | CHARACTERISTICS

UC Rio Zape produces seed that are nearly identical to its heirloom parent, with black stripes on a purple background (Figure 1). In the field, plants are large and indeterminate, with a type IIIb growth habit (Singh, 1982), green stems, and purple flowers. The average time to 50% flowering was 44 d after planting at Davis in 2019, whereas plots of the heirloom parent required 68–82 d to reach 50% flowering. UC Rio Zape is a full-season cultivar, and required at least 102 d to reach harvestable maturity at Davis in 2019, whereas Rio Zape needed more than 108 d. Seed color is consistent between locations. Like other beans with *C* locus-based stripe and spot patterns (e.g., pinto and cranberry types), a small percentage of UC Rio Zape seeds are reverse colored, with primarily dark purple or black, with lighter purple flecks on the seed (Bassett, 2007). These seeds are not off-types because their progeny revert to the usual color combination of black stripes on a purple background. Thus, there is no need to remove these reverse-color types unless the plants show other off-type characteristics, based on general experience with *C*-controlled variation in seed color pattern. Seeds darken after maturity, especially when stored under warm conditions. The subtle difference in seed color between UC Rio Zape and its heirloom parent in the Davis (2019 harvest) and Pescadero (2019 harvest) seed lots (Figure 1, picture taken shortly after harvest) is the result of the later maturity of the heirloom type, giving it less time to darken under warm field conditions. Note that seed lots from the San Juan Bautista field site of the previous year (2018) have stabilized to a similar color (Figure 1). Seeds that mature at the same time and are stored under the same conditions are not distinguishable, indicating that the *B* gene for seed color, which is tightly linked to *I*, is not segregating between cultivars. The 100-seed weight of UC Rio Zape is 47 g compared with 42 g for Rio Zape. No intentional selection for seed size was performed, so this difference is probably the result of BCMV resistance. Alternatively, there could be another locus linked to *I* that may be responsible for the variation in seed size.

In the multilocation field trials, UC Rio Zape had average seed yields of 1,504 kg/ha compared with Rio Zape's 1,260 kg/ha. This represents a yield improvement of 19%, which is statistically significant ($p = .018$, paired two-sample two-tailed *t*-test, Table 1). Although yield data deviated somewhat from a normal distribution ($p = .01$ for both types, Shapiro–Wilk test), *t*-tests are robust to even fairly major deviations from normality (Box, 1953; Edgell & Noon, 1984). UC Rio Zape's yield improvement relative to Rio Zape was relatively consistent across locations. In warmer climates such as Valley Center (July average high/low temperatures = 33/15 ˚C) and Davis (July averages = 34/14 ˚C), seed yields of UC Rio Zape averaged 789 kg/ha compared with Rio Zape's 638 kg/ha. This is an increase of 24%. UC Rio Zape also outperformed Rio Zape in the intermediate climate of San Juan Bautista (July averages = 27/12 ˚C), where it yielded 3,706 kg/ha compared with the heirloom's 3,197 kg/ha (+16%). Similarly, the coolest field site in Pescadero (July averages = 22/11 ˚C) averaged of 2,876 kg/ha for UC Rio Zape against Rio Zape's 2,427 kg/ha (+19%). This yield advantage makes UC Rio Zape a good alternative to Rio Zape across a range of climates. Despite this, the overall yield per area of both UC Rio Zape and its heirloom parent are far higher in cool- or intermediate-temperature environments, with poor yield stability in high-temperature organic environments. In the cooler environments of Pescadero and San Juan Bautista, the coefficient of variation for plot yield was 14% for UC Rio Zape and 16% for its recurrent parent. In the warm environments of Davis and Valley Center, this increased to 78% for UC Rio Zape and 121% for the recurrent parent. In these warm-season environments, both genotypes may benefit from long growing seasons and moderate terminal drought, which promotes a transition to reproductive growth and maturation. Similar patterns are seen in other race Durango landraces from the southwestern United States.

TABLE 1 Yield and *Bean common mosaic virus* (BCMV) comparison between UC Rio Zape and Rio Zape by year and location

Year	Location	Yield		BCMV observed[a]	
		UC Rio Zape	Rio Zape	UC Rio Zape	Rio Zape
		kg ha^{-1}			
2017	Davis	984	1,155	–	+
2018	Davis	182	0	–	+
	San Juan Bautista	3,706	3,197	–	+
	Valley Center	190	174	–	+
2019	Davis	1,170	239	–	+
	Pescadero	2,876	2,427	n.d.	n.d.
	Valley Center	1,420	1,624	–	+
Mean		1,504*	1,260	0%	100%

[a]+ indicates symptoms present; − indicates symptoms absent; n.d. indicates no data.
*Indicates significant difference ($p < .05$), paired two-tailed *t*-test of block data.

TABLE 2 Flavor and cooked appearance desirability of Rio Zape and UC Rio Zape (1–5 scale, where 1 indicates inferior and 5 indicates superior). Samples for taste test harvested in Davis, CA, 2018

Score	Rio Zape	UC Rio Zape
Flavor		
Mean	3.42	3.52
SD	0.89	1.03
SE	0.15	0.18
n	33	33
Paired *t*-test *p* value	.67	
Cooked appearance		
Mean	3.55	3.63
SD	1.05	1.17
SE	0.19	0.21
n	30	30
Paired *t*-test *p* value	.74	

The type ‘Anasazi’, for example, also shows strong vegetative growth until cool, dry conditions in fall cause a strong transition to maturity (Hang, Silbernagel, & Miklas, 2003; Silbernagel, Hang, & Miklas, 1998).

No UC Rio Zape plants developed BCMV symptoms at any field site in any year, whereas the disease was identified in Rio Zape at all evaluated locations (Table 1). UC Rio Zape did not differ from its heirloom parent in flavor and or cooked visual appearance ($p = .67$ and $p = .74$, respectively, paired two-sample *t*-test, Table 2), indicating no loss of culinary quality due to introgression of the *I* allele.

Like its heirloom parent, UC Rio Zape expresses a relatively viney type III growth habit, This trait may help with weed suppression and provide higher yields under certain conditions but may also be problematic in humid areas or for growers that use direct harvest. Under these conditions, it is recommended to evaluate UC Rio Zape on a relatively small scale before expanding production.

UC Rio Zape was developed using both genotypic and phenotypic selection. This allowed the combination of the strengths of each method (Beaver & Osorno, 2009). During successive rounds of backcrossing, for example, phenotypic selection ensured that recombination did not occur between the genetic marker and the causal gene. Similarly, the genetic marker provided a redundant backup for selections during backcrossing. After backcrossing, use of the co-dominant marker allowed for the identification of homozygous resistant individuals, which could not be readily distinguished from heterozygotes through phenotypic selection alone. This complementation of phenotypic and genotypic screening is likely to be a way forward for breeding in dry bean and other crops.

4 | AVAILABILITY

Breeder seed has been provided to the UC Davis Foundation Seed Program, which will produce and distribute seed for this cultivar. Small quantities of seed for breeding and research may be available through the corresponding author. Seed has also been deposited with the USDA–ARS National Plant Germplasm System, where it will be available immediately upon publication.

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AUTHOR CONTRIBUTIONS

Travis Parker: Parent evaluation; Methodology planning; Crosses; Field trial design and management; Genotyping; Phenotyping; Intern mentorship; Data analysis and selections; Manuscript writing; Funding acquisition. **Antonia Palkovic**: Parent evaluation; Field trial design and management; Intern mentorship. **E. Charles Brummer**: Conceptualization; Funding acquisition. **Paul Gepts**: Conceptualization; Parent evaluation; Methodology planning; Manuscript writing; Funding acquisition. All authors have read and approved the final draft of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Travis Parker https://orcid.org/0000-0002-1233-7829
E. Charles Brummer https://orcid.org/0000-0002-3621-4516
Paul Gepts https://orcid.org/0000-0002-1056-4665

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