

Research Article

Agronomic Evaluation of Bunching Onion in the Colombian Cundiboyacense High Plateau

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Received 4 October 2017; Accepted 29 November 2017; Published 1 January 2018

Academic Editor: Allen Barker

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Bunching onion (*Allium fistulosum* L.) is a strategic crop for Colombia due to its economic relevance within fresh and processed food markets, and therefore, there is a demand for high yielding genotypes adapted to specific regions. For this reason, after carrying out a clonal selection process including 62 genotypes, ten of these, including a regional control, were evaluated for six different traits in Boyacá (Colombia) during 2012 and 2013. These traits were grouped into agronomic, yield, and processing categories. In general, these showed significant differences ($p \leq 0.01$) for genotypes, location, and genotypes $\times$ location interaction. Compared with the regional control and based on the multienvironmental analysis the genotypes Clone 30 and Clone 38 were the most promising new cultivars identified in this study. These two clones showed comparative advantages on earliness and yield, and they moreover showed some level of resistance to downy mildew and root rot, the most limiting diseases for Boyacá's bunch onion farmers. Therefore, Clone 30 and Clone 38 were registered as new bunching onion cultivars for the Cundiboyacense High Plateau region under the names Corpoica Aquitania-1 and Corpoica Tota-1, respectively. Finally, further approaches and initiatives on bunching onion breeding are discussed.

1. Introduction

Within the *Allium* genus, bulb onion (*Allium cepa* L.), garlic (*A. sativum* L.), and bunching onion (*A. fistulosum* L.) are economically the most important species worldwide. Bunching onion, also known as Welsh onion or scallion, is one of the most important crops in Japan, China, and Korea. It is a diploid ($2n = 16$) and allogamous perennial species. The hypothesis on its place of origin suggests that bunching onion comes from northwestern China, but today it is widely adapted and cultivated around the world [1]. Therefore, the highest genetic diversity resources and formal breeding programs are mainly located in China and Japan. Bunching onion is mostly appreciated for its high nutritional value and unique flavor, due to its substantial amounts of vitamins, macro- and micronutrients, volatile oils, and flavonoids that also give it strong antioxidant properties [2, 3]. Moreover, roots, bulbs, and leaves of *A. fistulosum* have traditionally

been used in this region for the treatment of febrile diseases, headache, abdominal pain, and diarrhea and are widely used for their antifungal, antioxidative, antiplatelet, antihypertensive, and antiobesity activities [4–6].

Bunching onion is a typical outcrossing crop due to protandry [1]. It is self-compatible but susceptible to severe inbreeding depression [7]. Commercial F1 hybrid varieties are very important in countries that cultivate bunching onion. However, Colombia's production is based on clonal propagation due to its temperate origin and vernalization requirements for bolting [8]. Currently, bunching onion research has mostly been focused on yield [8], freezing tolerance [9], resistance to *Fusarium oxysporum* [10, 11], extraction of medicinal compounds [6, 12], and pungency reduction [13].

Although bunching onion is the second largest vegetable crop produced in Colombia with 17,000 hectares producing 327,000 tons, limited efforts have been made to focus on

TABLE 1: Experimental location used to evaluate bunching onion.

Trial	Town	Location	Latitude	Longitude	m.a.s.l.	Planting	Harvest
T1	Aquitania	Hato Viejo	5°31'34,198"N	72°52'31,227"W	3074	14/09/2013	28/12/2014
T2	Aquitania	Vargas	5°31'18,156"N	72°53'31,24"W	3015	26/04/2013	20/2/2014
T3	Tota	Toquecha	5°31'58,89"N	72°59'11,644"W	2773	10/07/2012	5/2/2014
T4	Tota	Cardón	5°31'30,121"N	72°58'31,332"W	3024	31/05/2013	12/02/2014
T5	Cuitiva	Llano de Alarcón	5°32'13,4"N	72°59'45,4"W	3020	10/07/2013	12/2/2014
T6	Aquitania	Hato Viejo	5°31'34,198"N	72°52'31,227"W	3074	10/07/2012	5/2/2014

crop improvement. In terms of genetic resources, the first initiative led by ICA was carried out around the mid-eighties where some accessions were collected, conserved, and characterized [14]. However, since then and due to inconstant public funding of research schemes and unclear agricultural research vision, no improved bunching onion cultivar has been introduced to any market so far.

Boyacá was the department that stood out during 2010 to 2015 with 70% of the national production, and the productive chain linked from small-scale farmers, to the agroindustry, transportation actors, and the final market [15]. Therefore, bunching onion is an important element for the region's rural development. However, the cultivated area and yield have over the years been reduced due to the incidence of pest and diseases. Consequently, efforts in developing high yielding cultivars with specific and broad adaptability, precocity, resistances to various pest and diseases, and good marketing quality are needed to support the national bunching onion production in the department and in the whole country. Additionally, improving agronomic practices is required; for example, the broadly used practice of using uncomposted chicken manure increases pathogen problems as root rot caused by the *Burkholderia cepacia-Ditylenchus dipsaci* complex, which is the most limiting problem in the Boyacá region [16].

For the reasons mentioned above, this research aims at the identification, evaluation, and selection of promising clones from the Colombian Germplasm Bank. The main goal is to identify elite materials adapted to the current bunching onion growing areas in Colombia, with tolerance to diseases and fitted to farmers and market preferences.

2. Materials and Methods

A total of 62 accessions of the Colombian Germplasm Bank were evaluated in six onion growing areas. Four clonal section cycles were carried out based on the Mamá and Bebé approach [17] (data not shown). Of these, nine clones (Clone 14, Clone 17, Clone 18, Clone 19, Clone 27, Clone 28, Clone 30, Clone 33, and Clone 38) and a regional variety known as "Pastusa" (control) were selected based on agronomic performance and farmers' preferences. The trials were carried out following the Colombian regulation for new cultivars using an Agronomic Performance Test supervised by Instituto Colombiano Agropecuario (ICA) according to resolution number 3168.

The trials were carried out during 2012 and 2014 in six common onion growing locations in towns near Lake

Tota in the Cundiboyacense High Plateau (Table 1). The experimental design was a random complete block with four repetitions. The experimental unit included four rows of 8 m with distances of 0.40 m and 0.80 m between plants and rows, respectively. The plants evaluated were measured from the two inner rows. Crop management, that is, fertilization, irrigation, and weeds, pests, and diseases control, was carried out according to the onion's technical manual elaborated by Sanchez et al. [16].

The agronomic traits evaluated per plant were leaf and pseudostem length (LL, SL) and diameter (LD, SD), days to flowering (DF), plant height (PH), number of flowers (FN), days to harvest (DH), and mildew and root rot severity (MS, RRS). Yield (Y) was evaluated as the sole yielding trait. The processing traits evaluated were dry matter content (DM), pyruvic acid content (APC), and flour and paste production; these analyses were carried out in the Fruits and Vegetables Pilot Plant of La Salle University (protocols available upon request).

For the statistical analysis of the data obtained, a combined variance analysis, a Tukey mean comparison, and the additive main effects and multiplicative interaction (AMMI) were carried out using SAS software v. 9 (SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Agronomic Traits. All agronomic traits evaluated showed significant differences between location, genotypes, and location $\times$ genotype interaction based on a variance analysis. Therefore, the environmental effects on the onion's agronomic traits were clearly identified. For instance, the trials T1 and T2 presented on average a higher leaf length value (43.6 cm) in comparison to T3 (16 cm) (Figure 1(a)). Clone 33 showed the highest leaf length value with 44 cm followed by the regional control (40 cm), Clone 38 (37 cm), and Clone 30 (37 cm). The genotype with the lowest leaf length values was Clone 19 (23 cm) (Figure 1(b)). Similarly, trial T5 showed the highest average in leaf diameter value (2.5 cm) in comparison with T3 (0.4 cm) (Figure 1(c)). Clone 33 and Clone 30 showed the highest leaf diameter values of 4.8 cm and 2.9 cm, respectively (Figure 1(d)). Leaf traits are important, despite the fact that these are not edible parts in Colombia, mostly because certain markets use them as visual signs of plant vigor and shelf-life. Interestingly, bunching onion leaves are extremely popular in the Asian cuisine and their nutritional properties are very well known [18]. Therefore, bunching onion leaves might have an alternative

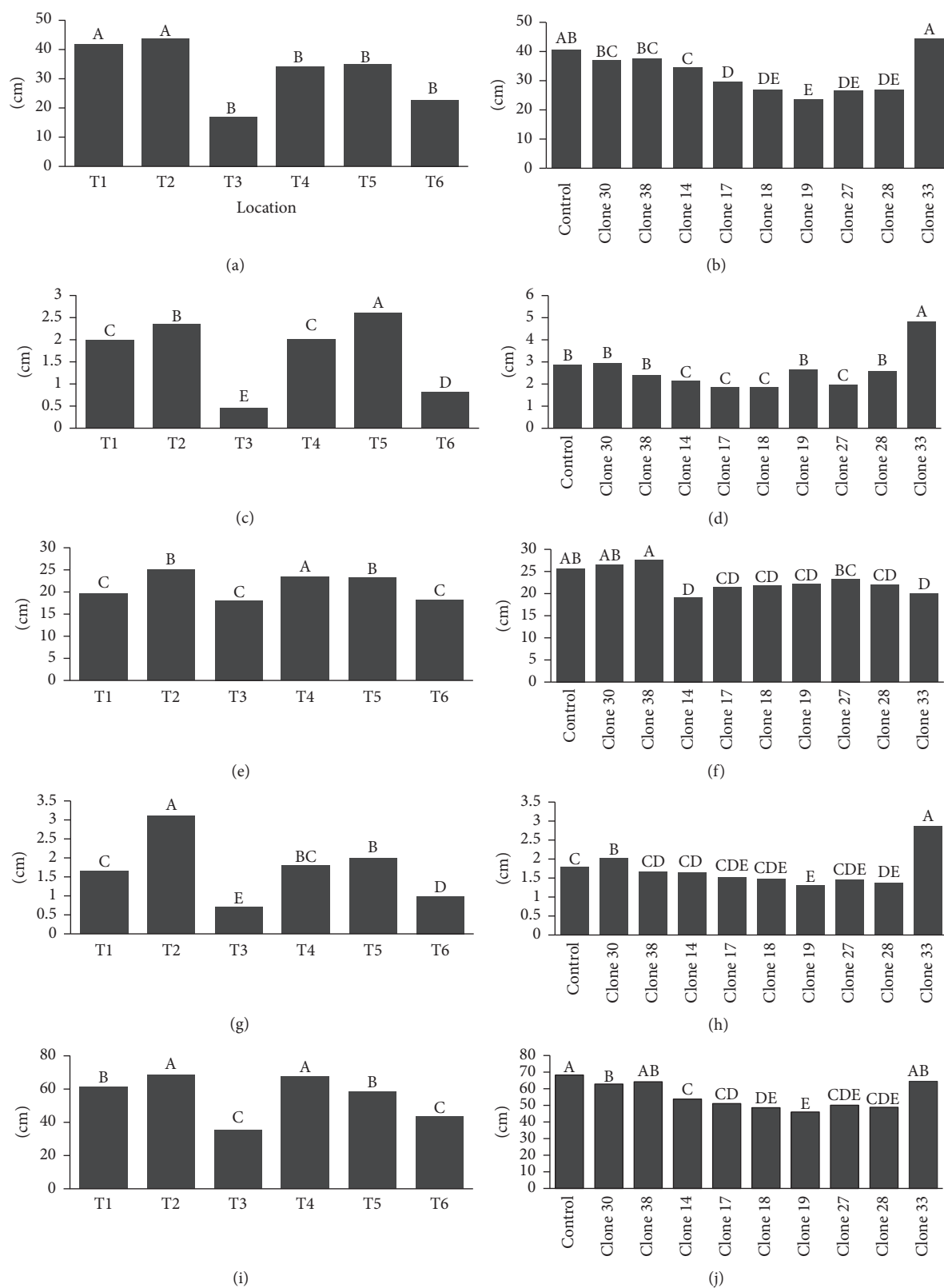


FIGURE 1: Agronomic traits evaluated for ten genotypes of bunching onion on six trials. (a) Leaf length by trials; (b) leaf length by genotypes. (c) leaf diameter by trials; (d) leaf diameter by genotypes; (e) pseudostem length by trials; (f) pseudostem length by genotypes; (g) pseudostem diameter by trials; (h) pseudostem diameter by genotypes; (i) plant height by trials; (j) plant height by genotypes.

and potential use for specific national and international niche markets.

On the other hand, pseudostem length and diameter are extremely important traits in the Colombian bunching onion markets, as the widest genotypes are marketed on local fresh markets, middle size ones are preferred by grocery stores, and the thinnest ones are used by the processing industry.

Trials T2 and T5 showed the highest average in pseudostem length values with 25 cm and 23 cm, respectively (Figure 1(e)). The lowest values were found in T1 and T3 with 18 cm and 17 cm, respectively. The genotype Clone 38 showed the longest pseudostem length value (27.64 cm) across all the locations, followed by Clone 30 (26.5 cm) and the regional control (25.6 cm) (Figure 1(f)). In terms of pseudostem diameter, the T2 trial showed the highest value (3.1 cm) in comparison to T3 (0.71 cm). Clone 33 showed the largest diameter length (2.8 cm) followed by Clone 30 (2.04 cm) and the regional control (1.79 cm).

Plant height values were consequently with leaf and pseudostem length data; therefore the trial T2 presented the highest average value and the tallest genotypes were regional control (68.2 cm), Clone 33 (64.3 cm), Clone 38 (64.1 cm), and Clone 30 (62.7 cm). Normally, farmers prefer higher plants, although the leaf/steam proportion is also very important. For instance, the regional control showed the highest plant length values and had a good leaf length proportion. However, Clone 38 and Clone 30 have, among others, the highest plant length values with an important pseudostem length.

Regarding flowering, in theory onion is a temperate crop requiring a vernalization phase for bolting. Therefore, in tropical conditions with short days, flowering is not expected. However, under specific stress conditions or after several harvest cycles the flowering becomes an important issue under Colombian conditions. Interestingly, Clone 38 and Clone 30 showed more days to flowering as well as a reduced number of flowers compared to the regional control (data not shown).

3.2. Yield and Processing Traits. Evaluation of days to harvest (DTH) showed that on average the trial with less days to harvest was T1 (180 days) in contrast to T3 (203 days) (Figure 2(a)). Similarly, genotype Clone 28 had the longest number of days to harvest (260 DTH) and Clone 14, Clone 30, and Clone 38 were the most precocious ones with 179 DTH, 172 DTH, and 162 DTH, respectively (Figure 2(b)). As bunching onion in Colombia is propagated asexually, the material with less days to harvest or which is precocious is preferred over the less precocious one because it allows harvesting several times during a year. Another advantage of the harvesting earliness is the possibility of escaping abiotic stress conditions such as drought or freezing, very common in onion cultivating areas in Colombia.

The trait yield showed significant differences between trials, genotypes, and genotype $\times$ location interaction. Trial T5 showed the highest yield value ($34 \text{ t} \cdot \text{ha}^{-1}$) and T1 showed the lowest one with $18 \text{ t} \cdot \text{ha}^{-1}$ (Figure 2(c)). Genotypes Clone 33, Clone 38, Clone 30, and the regional control were considered as high yielding materials with $32.7 \text{ t} \cdot \text{ha}^{-1}$, $31.8 \text{ t} \cdot \text{ha}^{-1}$,

$30.05 \text{ t} \cdot \text{ha}^{-1}$, and $28.8 \text{ t} \cdot \text{ha}^{-1}$, respectively (Figure 2(d)). Based on the average yield of $13 \text{ t} \cdot \text{ha}^{-1}$ calculated for bunching onion in Colombia, these materials are a very promising alternative to the ones currently used.

Pungency in these onions is a market-based trait depending on consumers' preferences and it is normally measured by pyruvic acid content [19]. The genotype with the highest pyruvate acid content was Clone 27 ($36,152 \mu\text{mol} \cdot \text{g}^{-1}$) and the one with the lowest value was the regional control ($21,717 \mu\text{mol} \cdot \text{g}^{-1}$) (Figure 2(e)). These values were found to be higher than in previous evaluations carried out in bulb onion by Wall and Corgan [19] and in bunching onion by Levine et al. [20] as a potential resource for the spices industry.

Similarly, the genotype with the highest dry matter content was Clone 19 (10.9%) and the lowest values were found in the regional control and Clone 38 with 7.66 and 7.43, respectively (Figure 2(f)). Additionally, the most efficient genotypes for paste and powder production were Clone 27 (48.1%) and Clone 19 (8.1%), respectively.

These results are interesting because fresh bunching onion market is very much an uncontrolled supply and demand scenario of an inelastic product; that is, there is always an uncertain price paid to farmers. Therefore, the onion agroindustry is an alternative fair-trade market for small-scale farmers, and as a consequence, the characterization and improvement of processing traits will open new market alternatives for small-scale farmers.

3.3. Genotype $\times$ Environment Interaction ($G \times E$). The additive main effects and multiplicative interaction (AMMI) analysis was carried out for yield and pyruvic acid, showing significant differences between environments, genotypes, and genotypes $\times$ environment. Specifically, the determination and variation coefficients for yield were 0.7% and 44.6%, respectively. The total phenotypic variation is explained by the location (52.9%), genotype (6.2%), and $G \times E$ interaction (15.1%). The proportion of the total variation accounted by each of the principal axes of the AMMI model was 71.9% for the first component and 12.1% for the second component.

Based on the graphical representation (Figure 3(a)), the genotypes Clone 17 and Clone 38 were located near to the axes showing a yield stability that was independent from the environment. Interestingly, this plasticity behavior under multiple environment trial evaluations has been identified as related to abiotic stress tolerance, suggesting that genes underlying plasticity could be related to stress response [21]. Additionally, specific adaptations were found to be related to particular trials. For instance, Clone 33, Clone 38, and the regional control showed particular adaptation to T6; and Clone 30 and Clone 14 were adapted to T1 and T3.

The $G \times E$ interaction analysis for pyruvic acid reported a determination coefficient of 0.8% and a variation coefficient of 19.5%. The total variation is explained by location (33.5%), genotype (30.5%), and $G \times E$ interaction (15.7%). The first and second principal components accounted for 53.2% and 33.7% of the phenotypic variance, respectively. In terms of stability, Clones 28, 33, and 38 showed the highest plasticity on pyruvic acid content. On the other hand, Clone 17 and Clone 28 showed specific adaptability to trials T1 and T6.

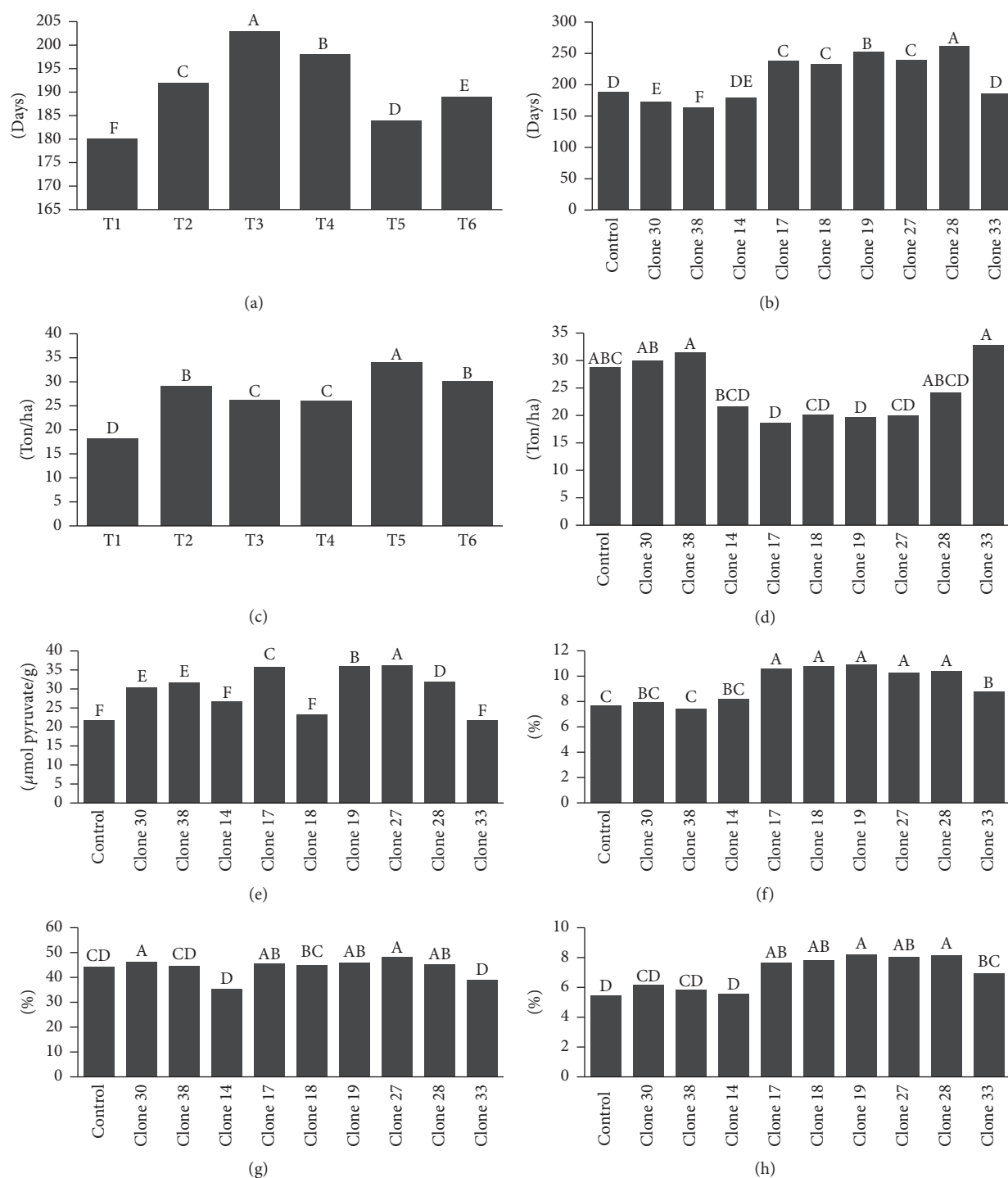


FIGURE 2: Earliness, yield, and processing traits were evaluated for ten genotypes of bunching onion. (a) Days to harvest (DTH) by trials; (b) DTH by genotypes; (c) yield by trials; (d) yield by genotypes; (e) pyruvate concentration; (f) dry matter content; (g) paste production (final paste weight/initial weight); (h) powder production (final powder weight/initial weight).

Interestingly, Clone 14, Clone 18, Clone 19, Clone 27, Clone 30, and the regional control presented a wide variation based on the contribution of each environment evaluated.

3.4. Diseases Evaluation. The most limiting bunching onion diseases in the Boyacá region are downy mildew (*Peronospora*

destructor) and the root rot (*Burkholderia cepacia-Ditylenchus dipsaci* complex). The results showed that the regional control is susceptible to mildew with a 49.1% of disease severity. Interestingly, the other genotypes evaluated presented moderate to high resistance. However, the most promising genotypes in terms of agronomic behavior

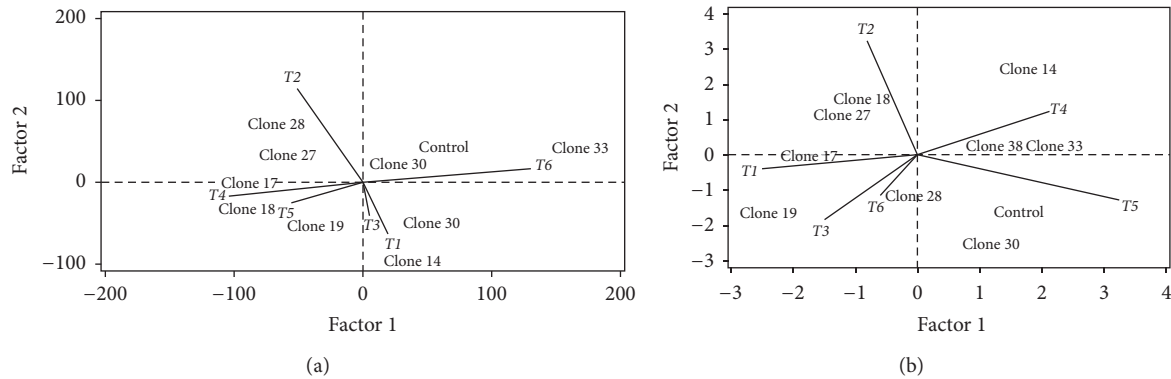


FIGURE 3: AMMI biplot for (a) yield and (b) pyruvic acid content showing genotypes and trials. Any two trials or genotypes are highly correlated if they are located at nearly the same angle from the center of the plot; it will be negatively correlated if opposing and nearly uncorrelated if the angle between them is close to 90 degrees.

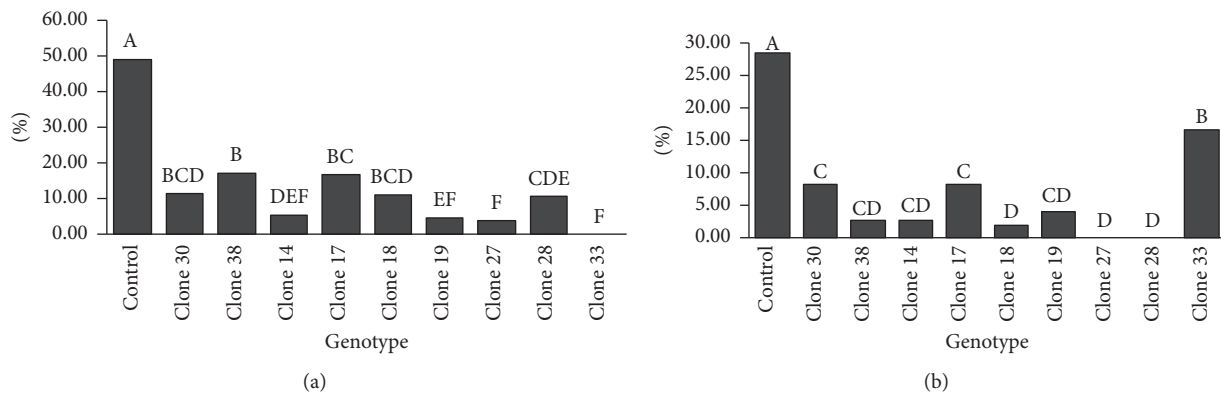


FIGURE 4: Evaluation of ten selected bunching onion genotypes for (a) downy mildew and (b) root rot disease severity.

were Clone 30 and Clone 38 showing 11% and 17% of disease severity, respectively (Figure 4(a)). The use of these promising clones in Colombia's onion growing area would influence directly the farmers' economy as it would reduce the supply costs (less fungicides) and would indirectly benefit the ecosystem by releasing fewer chemicals into the fragile environment of the Lake Tota basin. Similarly, the high severity symptoms for root rot were detected for the regional control with 28.4%; Clone 30 and Clone 38 showed moderate resistance with 8% and 2.7% of disease severity, respectively (Figure 4(b)).

These results revealed a promising variability within Colombian bunching onion germplasm kept in the Colombian Germplasm Bank. However, in order to continue the efforts on plant breeding there are some steps to be followed:

- (1) Germplasm collection, introduction, and characterization: there are some specific bunching onion types in the Colombian northern markets called "sonsonaña" that are unrepresented in the germplasm bank, and therefore, germplasm collecting missions have to be carried out to include this and other genotypes still not included. Additionally and based on the fact that Colombia is neither the center of origin nor diversity of *A. fistulosum*, new germplasm has to be

introgressed from other germplasm banks or breeding programs around the world, especially from Asia [22]. Finally, the morphological characterization can be well complemented with genotyping information based on the increased availability of genomic tools such as SSR [23, 24] and transcriptome assembly [25].

- (2) Seed propagation initiatives: most of the Colombian bunching onion phytosanitary problems are due to poor asexual seed quality. Therefore, physiological and genetics studies have to be carried out to simulate long day conditions and vernalization based on high environmental effects on bolting [8].
- (3) Nutritional value: based on the functional food market tendency, bunching onion plays an important role due to its high vitamin C, carotenoids, macro- and micronutrients content, especially calcium and potassium, and flavonoids that are strong antioxidants [2, 3]. Therefore, these traits as well as their $G \times E$ interaction have to be evaluated and offered as a specific and additional value for specific onion markets.
- (4) Crop management studies: bunching onion crops in Colombia still have a knowledge gap in terms of nutritional requirements and disease management.

Therefore, multidisciplinary team efforts are necessary to tackle these needs in face of climate change challenges. Additionally, evaluation of other crop systems has to be carried out based on the evidence that greenhouse bunching onions showed yield improvements [26].

- (5) Breeding program: a long term initiative, preferably in joint collaboration with different institutions working on this species, has to be developed in order to cross and select recurrently families and genotypes with high frequency of favorable alleles. Additionally, intraspecific crosses and subsequently backcrossing schemes will incorporate well known disease resistance from other species such as *A. cepa* [10, 11]. Finally, the incorporation of cytoplasmic male sterility (CMS) using marker assisted selection [27] will accelerate the hybrid evaluation and trait introgression for new varieties.

4. Conclusions

These results are the first approach carried out in Colombia to offer new and potential bunching onion cultivars to farmers. Therefore and based on their wide adaptation, biotic stress tolerance, earliness, and yield, genotypes Clone 30 and Clone 38 were selected as new cultivars to be released in the Cundi-boyacense High Plateau and have been registered as Corpoica Aquitania-1 and Corpoica Tota-1, respectively. However, activities that follow the cultivars adoption in the current cultivation areas are ongoing and altogether will the beginning of a bunching onion breeding program in Colombia.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors are grateful to the farmers' associations Asoparcera, Asollanos, and Asotoquecha for their kind collaboration and motivation to carry out this study. They thank Juan Clímaco and Jorge Arguelles for their advice and support on the development of the phytopathological and statistical issues, respectively. This research was financed by the Colombian Ministry of Agriculture and Rural Development.

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