

Phenotypic Variability in the Induction of Alpha Acids in Hops (*Humulus lupulus* L.) in Brazil

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Abstract

Hops (*Humulus lupulus* L.) is a key ingredient in beer, with great significance for the Brazilian industry. The economically viable production of hops in Brazil depends on the genetic variability available to be used in the selection and development of high-as performance photo-neutral cultivars and their interaction with the environment. The objective of this research was to evaluate the phenotypic variability of alpha-effective character in relation to the environmental variation in different Brazilian regions. Alpha-acids tests were performed through the American Society of Brewing Chemists (ASBC-HOPS 6. B-Conductometric titration methodology). Phenotypic variation assessment showed that 30% of the variation occurred among clones, while 47% was attributed to the environmental components of cultivation. The repeatability coefficient (t) was below 0.25, demonstrating that interaction between genotype and environment inflicted on alpha-acid levels. Results showed variability in the alpha-acid contents depending on the hops growing regions. According to the results, clones 9 (8.22%), 26 (12.95%) and 27 (9.94%) added the highest levels of alpha-acids. Therefore, there is variability available for the genetic improvement of the culture in Brazil and its effects must be evaluated in each microclimate of cultivation.

Keywords: Alpha acid, genotype, genetic improvement

1. Introduction

Hops (*Humulus lupulus* L.) is an allogamous, perennial, native to Asia plant and from the Cannabaceae family (Boutain, 2014; Hieronymus, 2012). Their flowers or female strobili produce lupulin, contains resins, phenolic compounds, and essential oils (Durello et al., 2019). The most important chemical compounds of hops are alpha and beta acids, which give taste, aroma, and beer microbiologic stability (Durello et al., 2019; Almaguer et al., 2014).

The major hops producers worldwide are the United States (23.000 tons) and Germany (19.000 tons) (Hop Growers of America, 2019). Recently, hops production has been getting the attention of Brazilian farmers and breweries due to their strong economic potential (CERVBRASIL, 2016; Sebrae, 2014). Brazil is still not self-sufficient in hops production and imports around 4.000 tons/year (200 million reais a year) (Vendemiatti, 2020).

The lack of hops production on a Brazilian commercial scale is due to the cultivar agro-climatic zoning that did not apply to regions of tropical and subtropical climates (Dodds, 2019; Radtke, 1999). Hops are sensible to photoperiod, so this species has better adaptation to mild weather in regions 35° and 55° North or South latitudes because of the hot days, cold nights and light upper to 15 hours. The less quantity of light in regions close to the equatorial line does not allow expressing vegetative development in plants appropriated to high hops production (Krebs, 2019; Dodds et al., 2019).

However, through a set of agricultural practices, it is possible to increase productivity and enable hops cultivation in low-latitude regions. According to Brits (2008), the main techniques are irrigation, fertilization, artificial light,

and crop genetic improvement. South Africa has been selecting and adapting photo-neutral genotypes for subtropical environments for 60 years (De Lange et al., 2015; Beverley, 2015; Brits, 2008). In Brazil, the studies are still young and at the early stages of implementation. There are initiatives for crop genetic improvement in UDESC/CAV (University of Santa Catarina State), located in Lages-SC, in Universidade Estadual Paulista (UNESP) in Jaboticabal-SP, and in Cedral-SP at HOPs Brazil.

For crop improvement, a population with wide genetic variability needs to present good genes for the desired characteristics and the knowledge of their interaction with the environment (Allard, 1999). In Brazil, there are 50 genotypes available for purchase. Genotypes were selected in their native countries according to alpha-acids levels and their role in beer brewing: genotypes with alpha-acids levels from 5 to 20% are added to the wort boiling to check bitterness on dry hopping, and genotypes with alpha-acids levels from 0 to 7% are used to get taste and aroma to beer. Therefore, genotypes are classified in taste (0 to 7% of alpha-acids), dual-purpose (5 to 7% of alpha-acids) and bitterness (7 to 20% of alpha-acids).

Nevertheless, there is a high weather and soil conditions variability in Brazil, and the phenotypic change of the cultivars under Brazilian environment conditions is unknown (Bizotto, 2019; Chagas & Garcia, 2018; Radtke et al., 1999). The evaluation of genotype/environment interaction is crucial to optimize improvement strategies so reducing the interaction effect or, on the other hand, exploring their positive effects better (Vencovsky & Barriga, 1992).

As set out above and due to the lack of preliminary studies, this research was performed to quantify alpha-acid levels of hops clones and verify phenotypic variation under different crop environments in Brazil, aiming at genes selection.

2. Method

2.1 Data Source

Data were supplied by Escola Superior de Cerveja e Malte (College of Beer and Malt), located at Blumenau, Santa Catarina, Brazil. Alpha-acid rates were obtained from 28 clones with approximately 2-years of cultivation, distributed for 7 States and planted in 29 unicipal districts of Brazil (Table 1).

Table 1. The Brazilian States and their municipal districts where hops were cultivated for following alpha-acid chemical analyses

Brazilian State	Municipal Districts
Distrito Federal (DF)	Brasília
Minas Gerais (MG)	Araxá
Paraná (PR)	Ponta Grossa, São José dos Pinhais, Campo Largo
Rio de Janeiro (RJ)	Nova Friburgo, Niterói
Rio Grande do Sul (RS)	Gramado, Carlos Barboza, Nova Roma do Sul, Serafina Corrêa, São Francisco de Paula
Santa Catarina (SC)	Braço do Norte, Campo Alegre, Taguaí, Urubici, Urupema, Lages, Palmeira, São Joaquim
São Paulo (SP)	Sorocaba, Boituva, Vinhedo, Holambra, Itapetininga, Jaguariúna, Mogi das Cruzes, Santo Antônio do Pinhal, São Bento do Sapucaí

2.2 Chemical Analysis Method

The dry seed cones (10% humidity) were broken into samples of 100 grams, vacuum-packed in plastic bags and immediately sent Escola Superior de Cerveja e Malte. For performing the chemical analyses, the official methodology of American Society of Brewing Chemists was used ASBC-HOPS 6. B-Conductometric Titration.

2.3 Statistical Analysis

To quantify the various sources, it was used variance compounds obtained through data covariance, evaluated by the mathematic model:

$$Y_{ij} = \mu + \text{cln}_i + \text{Status}(\text{cln})_{ij} + e_{ij} \quad (1)$$

where, Y is values of the observed alpha-acids; μ is the general average. $\text{Status}(\text{cln})_{ij}$ is the clone_i effect aligned with the status effect; e_{ij} is the experimental error effect.

Intraclass correlation coefficient (t) was calculated from the variance compounds described by Fisher (1925) apud Gonçalves and Fritsche-Neto (2012), being a data reliability measurement using the formula:

$$t = \frac{\sigma^2 S}{\sigma^2 S + \sigma^2 W} \quad (2)$$

where, S is the estimation of the variance component balance among parcels, and W is the estimation of the variance component inside the parcels added to the residual variance component.

Averages were submitted to multiple comparison test DMS (Tukey-Kramer) (5% of probability), and all statistical analyses were performed using software SAS (SAS *Ondmands for academics*), through proc Mixed procedure.

3. Results

The analyses of alpha-acids phenotypic variance components for 28 clones evaluated in 7 Brazilian states showed variation among clones (σ^2_e : 1.88). However, variation inside the clones was twice as higher (σ^2_d : 3.19) than variation among clones, corresponding to 47% of the total variance. Data also showed intraclass correlation coefficient (0.25) was lower (Table 2).

Table 2. Intraclass correlation coefficient (t) for alpha acids (%), calculated on variance components estimation among clones (e) and into clones (d)

FV	Alpha-acids (%)
Among (e)	1.88
Into (d)	3.19*
Residue	2.23
T	0.25

Note. * Significant at 5% probability of error for test Z .

It is possible to verify the evaluation and quantification of alpha-acids in Table 3. Values for alpha-acids indicated high amplitude (11.39%) among clone's averages cultivated in Brazil. Lower values of alpha-acids were observed for clone 6 (6 = dual purpose) with 1.56% and clone 9 (9 = sourness) with 8.90%, clone 27 with 9.94% (27 = dual purpose) and clone 26 with 12.6% (26 = dual-purpose) showing the best performance. Values higher than 7% of hops alpha-acids are satisfactory for the brewing industry, giving the correct bitterness to beer (Hieronymus, 2012).

Table 3. Quantification average alpha-acids values from 28 clones cultivated in Brazil of hops in % (100 g of dried hops)

Clones	Average	Clones	Average	Clones	Average	Clones	Average
1	3.81	8	2.92	15	2.52	22	3.92
2	4.24	9	8.22	16	2.51	23	5.90
3	3.54	10	2.99	17	4.54	24	1.95
4	5.11	11	1.56	18	2.12	25	4.69
5	3.89	12	3.01	19	2.54	26	12.95
6	2.82	13	3.45	20	2.21	27	9.94
7	2.55	14	3.28	21	2.77	28	2.47

According to the MSD (minimal significant difference) average test 66 combinations or 18.3% of the evaluated clones showed significant differences, so confirming the variance compounds analysis showing phenotypical variability (Table 4).

Table 4. Estimation differences of 28 hops clones by multiple comparison MSD (Tukey-Kramer) test

n.	Cl	Cl	Estimation	Pr	n.	Cl	Cl	Estimation	Pr
1	1	26	-9.14	0.01	34	11	23	-4.33	0.04
2	1	27	-6.13	0.02	35	11	26	-11.39	0.01
3	2	26	-8.71	0.01	36	11	27	-8.38	0.01
4	2	27	-5.70	0.03	37	12	26	-9.94	0.00
5	3	26	-9.41	0.01	38	12	27	-6.93	0.01
6	3	27	-6.40	0.01	39	13	26	-9.50	0.00
7	4	26	-7.84	0.01	40	13	27	-6.49	0.01
8	4	27	-4.83	0.04	41	14	26	-9.67	0.00
9	5	9	-4.32	0.02	42	14	27	-6.66	0.01
10	5	26	-9.05	0.01	43	15	23	-3.37	0.04
11	5	27	-6.04	0.04	44	15	26	-10.43	0.00
12	6	9	-5.39	0.02	45	15	27	-7.42	0.01
13	6	26	-10.12	0.01	46	16	26	-10.44	0.00
14	6	27	-7.11	0.03	47	16	27	-7.43	0.00
15	7	9	-5.66	0.06	48	17	26	-8.41	0.00
16	7	23	-3.24	0.02	49	17	27	-5.40	0.05
17	7	26	-10.39	0.01	50	18	26	-10.83	0.00
18	7	27	-7.39	0.01	51	18	27	-7.82	0.01
19	8	9	-5.29	0.01	52	19	26	-10.41	0.00
20	8	23	-2.97	0.03	53	19	27	-7.40	0.01
21	8	26	-10.02	0.01	54	20	26	-10.74	0.00
22	8	27	-7.01	0.02	55	20	27	-7.73	0.01
23	9	11	6.66	0.01	56	21	26	-10.18	0.00
24	9	15	5.70	0.01	57	21	27	-7.17	0.01
25	9	16	5.71	0.03	58	22	26	-9.03	0.00
26	9	18	6.10	0.02	59	22	27	-6.02	0.01
27	9	19	5.68	0.03	60	23	26	-7.05	0.00
28	9	20	6.01	0.21	61	24	26	-11.00	0.00
29	9	21	5.45	0.04	62	24	27	-7.99	0.00
30	9	24	6.27	0.02	63	25	26	-8.26	0.00
31	9	28	5.75	0.03	64	25	27	-5.25	0.05
32	10	26	-9.96	0.01	65	26	28	10.48	0.00
33	10	27	-6.95	0.01	66	27	28	7.47	0.01

Note. * n = number; Cl = clone; Pr = probability.

4. Discussion

According to the results (Table 2) there is an important phenotypical variability, and this one is the primary material for hops genetic upgrading in Brazil (Cardellino & Rovira, 1987). Values achieved in Tables 3 and 4 (clones 9, 26 and 27) were the highest, expressing potential usage as breeders in cross-breeding blocks to get cultivars with a high concentration of alpha-acids (Yakima Chief, 2016).

However, phenotypical variation among clones' averages can be associated with reproductive, evolution (environment adaptation), and selection (natural or artificial) of environment-genotype interaction processes (Hieronymus, 2012; Darby, 2005). Most of the evaluated clones (56%) previously has already gone through improvement and they were selected to give taste and aroma to beer. In despite of the environment, they showed low percentage of alpha-acids (Hieronymus, 2012; Haunold, 1981). Most of the evaluated clones (56%) previously has already gone through improvement, and they were selected to give taste and aroma to beer. Despite the environment, they showed a low percentage of alpha-acids (Hieronymus, 2012; Haunold, 1981). However, phenotypical variation among clones' averages can be associated with reproductive, evolution (environment adaptation), and selection (natural or artificial) of environment-genotype interaction processes (Hieronymus, 2012; Darby, 2005).

Clones' dissimilarity can be due to the ploidy level since one of the strategies used in hops upgrading is the achievement of triploid clones (Nesvadba et al., 2017; Haunold, 1981). According to Borém et al. (2013), Trojak-Goluch and Skomra (2018), clones showing higher chromosomes number are stronger and better adapted to extreme weather conditions (higher temperatures and low photoperiod). Due to the formation of excessive pollen, however, it occurs a high mortality rate following unbalanced meiosis, which can lead to reproduction problems on triploids clones, so impeding cross-breeding processes.

The best performances for clones 9, 26 and 27 can be related to the environment effect, which could have been strengthened by the local weather where they were cultivated since they were evaluated in few places ($N = 1$) compared to the other clones ($N = 9$). The higher variation into the clones (3.19) and the low intraclass correlation ratio or the upper limit of heritability (0.25) indicate the complex genotype \times environment interaction. For Allard (1999), the higher the number of genotypes and environment, the more complex the interaction. Alpha-acids are chemical compounds from the secondary metabolism of plants as well, and that control them and; in addition, the environment strongly intervenes in the regulation of their gene expression (McAdam et al., 2013). The complex genotype and environment interaction occur because the environment can turn genes off or recruit them, promoting the development of several phenotypic characteristics, resulting in phenotypic variability (Falconer, 1981; Mcdan et al., 2014; Borém et al., 2013). Coimbra et al. (2008) assume the greater variation sources among quantitative characteristics of *Phaseolus vulgaris* L is the genotype \times environment interaction. Similar results on alpha-acids production in hops crop were found by Forteschi et al. (2019), underlining that the cultivar Cascade produced in Italy showed different performance (variation of 4% in alpha-acids values) because of the region (Alghero, Orosei and Domusnovas) and the planting season (2017, 2018, 2019).

Experiments from McAdam et al. (2014) evaluated a collection of 108 clones from several European regions. They concluded that the genotype/environment interaction significantly influences the percentage of alpha-acids produced by hops clones. Similar results were observed in the studies of Green et al. (1997), estimating the access Tettnanger produced in the United States compared to the one grown in Germany (alpha-acids values of 4,15 and 5,04%, a range of 18%).

From genetic improvement, the complex genotype/environment interaction can complicate selection since access showing good performance in an environment could be poor or bad in other places (Cardellino & Rovira, 1987). In the presence of a strong genotype/environment interaction effect, the researcher may adopt practices to reduce the effect of the interaction or explore its benefits. For hops, regardless of the hops clone (for bitterness, dual-purpose or aroma) in beer brewing (wort boiling and dry hopping), the substances of hops alpha and beta acids can affect beer bitterness and aroma. Consequently, it is crucial to examine the terroir of hops growing in Brazil through sensory, qualitative analysis associated with the weather conditions of the farming place. According to Van Holle et al. (2017), the soil type and agricultural practices, which establish the unique and exclusive characteristics of taste and aroma, whatever the user access or cultivar.

Overall, the improvement and selection of hops cultivars in Brazil should focus on genotypes linked to or exploit taste and aroma to the full (alpha-acids $< 7\%$). Because of the demand and the under-explored Brazilian market, they can generate new aromatic profiles with higher benefits. It is important to note, then a significant increase in artisanal breweries has been observed, and many consumers are more inclined to innovate on that market (Sebrae, 2020).

To decrease the effects, it is possible to use environment stratification. It is recommended to regionalise improvement programs, with cultivars development and selection for each cultivation microclimate (Allard, 1999). Environment stratification with better uniformity can make possible the development and cultivars recommendation. Environments with little instability are particularly advisable. A second alternative is the choice of phenotypically more stable cultivars through the development of genotypes or multiline cultivars, with greater adaptability, phenotypic balance, and positive response to environmental improvement (fertilization and watering).

5. Conclusion

There was phenotypic variation among clones of hops grown in Brazil (11% of amplitude), and part of the variation was assigned to genetics. Clones 9, 26 and 27 showed the best performance, and, this way, they should be used in breeding blocks to improve hops in Brazil. Therefore, there is variability available for the genetic improvement of the culture in Brazil and its effects must be evaluated in each microclimate of cultivation. Emphasis has to be intended on the selection and creation of clones with new tastes and aromas, resulting in a regional beer with regional identity and greater added value.

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