# Stability and adaptability of lettuce cultivars on different substrates

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## Abstract

Lettuce is a leafy species cultivated in different regions of the world. During the seedling production process, the choice of the substrate is one of the most important steps. Considering diversity of type and price, it is common to produce alternative substrates using inputs with potential to cause environmental impacts. Sludge is a waste from water treatment plants, which has agricultural potential. This research aimed to estimate the stability and adaptability of five lettuce cultivars under different substrate compositions in the early stages of growth and yield. Two experiments were carried out in 2021: 1) in greenhouse, in which five cultivars ('Veneranda', 'Camila', 'Elba', 'Vitória Verdinha', 'Diva'), three substrates ('Vida Verde', Bioplant 434, Bioplant 401) and three sludge doses (0, 20, 40%) were tested on the seedling quality index (SQI); 2) at field level, the yield of seedlings produced in the 1<sup>st</sup> stage was evaluated. From a double-entry table (matrix M), consisting of five cultivars and nine environments (combination of 3 substrates x 3 sludge doses), deviance analysis, WAASB and ASV stability indexes, biplots of the AMMI method and BLUPs were applied. Significant GE interaction identified cultivars adapted to specific substrates. 'Camila' cultivar was stable in terms of SQI and yield; 'Diva' was the most unstable, but with specific adaptation to five substrate for SQI and three for yield. The addition of 40% of sludge reduced the SQI; however, it did not influence the yield when associated with 'Vida Verde' and Bioplant 434, being indicated for production of lettuce.

Keywords: AMMI, BLUP, mixed models, WAASB, WTP sludge

## Introduction

The genus *Lactuca* spp. (Asteraceae) has, approximately, 100 species that stands out for its edible leaves, such as lettuce (*Lactuca sativa* L.), which has its origins in Asia (Vries, 1997). It is a short-cycle herbaceous species that can be cultivated in protected environments or at field level. It has  $C_3$  photosynthetic metabolism (Zhou et al., 2020), in which the ideal culture temperature varies between 15 and 18°C (Brunini et al., 1976). However, due to the presence of thermotolerant genes (Wei et al., 2021), it was possible to select resistant genotypes (Nascimento et al., 2012), making its cultivation viable almost all over the world.

After considering the genetic characteristics of the cultivar and seed quality, the choice of the substrate is the main step, as it is the input that is directly linked to the final quality of seedlings, with effects on commercial yield (Oliveira & Panno, 2011). The substrate must be

## pathogen-free

and have chemical and physical composition, capable of providing water, air and nutrients that enable rapid germination/emergence and seedling development (Salvador et al., 2013).

In Brazil, there is a variety of substrates available on the market, following the specifications established by Normative Instruction No. 5/2016 of the Ministry of Agriculture, Livestock and Supply. The differences observed are in composition, which involves the use of vegetable products (coconut fiber, rice husk, pine, peat), organic products (manure, organic compounds, earthworm humus), vermiculite, addition of synthetic fertilizers and growth-promoting microorganisms (Guto & Silva, 2020).

Substrate is an important factor that directly impacts production costs, along with freight. For this reason, the development of alternative substrates

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using agro-industrial residues present in the region is not uncommon (Correa et al., 2019, Zuffo et al., 2020). This practice, in addition to reducing costs, transforms disposable and polluting materials into value-added coproducts (Teodoro & Pereira, 2021), preserves natural resources, becoming an ecologically correct activity, by mitigating environmental damage, in addition to increasing the useful life of landfills.

In water treatment plants (WTP), sludge is obtained as waste, classified as Class IIA, non-inert, with biodegradability, combustibility or water solubility properties (ABNT, 2004). According to Gonçalves et al. (2017), WTP sludge is mostly composed of inorganic fractions (clay, silt and fine sand), as well as organic material and microorganisms, often from the siltation of rivers. These characteristics enable its use as a component in agricultural studies.

Some studies have shown that sludge has potential in the composition of substrates, such as that developed by Cunha et al. (2020), in the production of eucalyptus seedlings, who observed that the addition of up to 45% of WTP sludge incorporated into the soil and sand contributed to seedling germination and growth. Brito et al. (2021) observed that the composition of 75% WTP sludge and 25% commercial substrate promoted higher quality indexes of favela seedlings (*Cnidoscolus quercifolius* Pohl.). The different compositions of substrates may promote different effects on seedling germination and development, depending on the species and cultivar.

In plant breeding, phenotype is a function of genotype, environment and the genotype x environment (GE) interaction, considering the environment (biotic, abiotic and management practices) factors capable of influencing the phenotypic behavior (Gull et al., 2019). In this concept, it is understood that substrates are environments that can influence, positively or negatively, seedling development. Therefore, selecting genotypes that express maximum genetic potential in a specific substrate will bring more efficiency to the production process. The analysis of stability and adaptability allows us identifying this relationship, exploring the GE interaction.

There are several biometric models that are used to estimate adaptability and stability (non-parametric, uni and multivariate). Computational evolution made it possible for complex mathematical models to be incorporated into methods, such as the WAASB index, developed by Olivoto et al. (2019). Based on mixed models, this index combines the characteristics of the additive model and the multiplicative interaction of the AMMI method with the predictions of the BLUP method. Intuitive interpretation in biplots has become a popular tool, allowing the identification of genotypes that show consistent behavior in multi-environments (Yan et al., 2007).

Despite being presented as a new method, the WAASB index has been efficiently adopted in different species, such as soybean (Nataraj et al., 2021), barley (Verma & Singh, 2021) and wheat (Aboughadareh et al., 2021). However, in leafy species, such as lettuce, there are no works published in national and international literature. For this reason, this research aimed to estimate stability and adaptability parameters of five lettuce cultivars under different substrate compositions at the early stages of growth and yield.

## **Material and Methods**

The research was carried out in Arapiraca, Alagoas – Brazil, tropical savanna climate, 'Aw' classification according to the Köppen classification (Climate-Data, 2022), and was divided into two stages: a) greenhouse, aiming to evaluate the plants at the initial stages; b) production field, aiming to evaluate the point of harvest/marketing.

In the 1<sup>st</sup> stage, seedlings were produced in greenhouse for commercial seedling production, chapel model, covered with low density polyethylene film, located in the district of Flexeiras, Arapiraca/AL, with geographic coordinates -9.799041 S and -36.604969 W, at 229 m a.s.l., between February and March 2021. A digital thermo-hygrometer equipment, model FEPRO-MUT60OS, was used to monitor variations in temperature and relative humidity inside the greenhouse for 14 days after sowing (Figure 1).

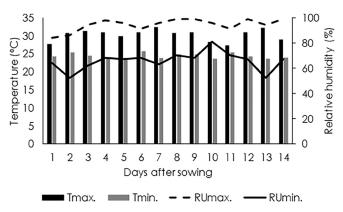


Figure 1. Temperature (T) and relative humidity (RU) variation in the greenhouse.

From the 15<sup>th</sup> day onwards, seedlings with three leaves underwent the adaptation process, outside the greenhouse (full sun). At 25 days after sowing, they were able to transplant with six leaves. The average temperature, minimum and maximum, relative humidity and precipitation during this period (10 days) were 22.7 and 34.8°C, 67.7% and 45.8 mm, respectively.

The experiment was installed in a completely randomized design, 5 x 3 x 3 factorial arrangement, with five lettuce cultivars ('Veneranda', 'Camila', 'Elba', 'Vitória Verdinha' and 'Diva'), three commercial substrates for vegetable (Vida Verde®, Bioplant 434® and Bioplant 401®) and three sludge levels (0, 20 and 40 of WTP sludge replacement by commercial substrate), with four replicates. The plot was composed of 10 seedlings, considering the eight central ones as useful area.

Lettuce cultivars were selected considering their wide acceptability by producers and consumers and have the following characteristics: 'Veneranda', crispi type, leaf edges chopped green in color; 'Camila', large light green leaves; 'Elba', crispi type, large light-green leaves; 'Vitória Verdinha', smooth type, without head formation, intense green color; 'Diva', crisphead type, intense green color.

The technical information, provided by substrates manufacturers, were: 'Vida Verde', composed of rice husk and pine, coconut fiber, pH 5.8, super simple, potassium nitrate, 14-16-18 formula (NPK), Yoorin Master, electrical conductivity 0.5 dS m<sup>-1</sup>, water holding capacity of 150%; Bioplant 434, consisting of Sphagnum peat, vermiculite, coconut fiber, rice husk, pH 6.5, limestone, 11-52-00 formula (NPK), electrical conductivity of 0.8 dS m<sup>-1</sup>, water holding capacity of 100%; Bioplant 401, varies only in relation to the 14-16-18 formula (NPK).

The sludge from the 'São Francisco River' water treatment plant was obtained from company 'Agreste Saneamento S/A' in January 2021. It was then dehydrated in an oven at 65°C until reaching constant mass and, subsequently crushed in a disk mill (Botini®). Samples were analyzed following the NBR 10004:2004 standard, in which the concentrations of toxic organic and inorganic residues are absent or below the maximum levels allowed, without risk to human health.

From a sample composed of WTP sludge, formed by 10 simple samples, chemical analyses were carried out, indicating: pH (water), 6.1; P (Mehlich), 1.0 mg dm<sup>-3</sup>; K and Na (Mehlich), Ca, Mg and Al (1N KCl), H (calcium acetate pH 7.0), 0.25, 0.25, 4.0, 2.9, 0.0, 2.7 cmol<sub>c</sub> dm<sup>-3</sup>, respectively. The total organic matter content was 13.1%. The Fe, Cu, Zn and Mn (Mehlich) contents were 376.0, 0.90, 1.80 and 302.1 mg dm<sup>-3</sup>, respectively. Physical analysis showed that the sludge is classified as clayey loam, with the following constitution: coarse sand, fine sand, silt and clay of 214, 114, 364 and 328 g kg<sup>-1</sup>, respectively.

Seedlings were produced in 200-cell trays, with volume of 18 cm<sup>3</sup> each, manually placing one pelleted seed per cell. In all treatments, Carolina Soil® commercial substrate was used only to cover seeds, as it presents higher vermiculite content, greater moisture retention and allows the same emergence condition. Irrigation was carried out via microsprinkler, using the Gyronet LR Netafim system, flow rate of 70 L h<sup>-1</sup>, with application of water depth of 1.6 mm for approximately 40 minutes. These management practices are adopted in the region at the commercial production level.

Twenty-five days after sowing, using eight plants from the useful area of the plot, the average seedling height (SH, mm) was evaluated, measured from the root collar to the apex of the seedling, with the aid of a ruler; stem diameter (SD, mm), measured on the seedling collar with the aid of a caliper; shoot mass (SM, g) was measured with the aid of digital analytical scale; root mass (RM, g), obtained after separating the root from the substrate, in running water; total mass (TM, g), obtained by summing up the shoot and root masses. Using these data, the seedling quality index (SQI) proposed by Dickson et al. (1960) was calculated, where:

$$SQI = \frac{TM}{\frac{SH}{SD} / \frac{SM}{RM}}$$

Seedlings produced in the 1<sup>st</sup> stage were taken to the field (2<sup>nd</sup> stage) on a private property located in the district of Batingas, Arapiraca/AL, geographic coordinates -9°79'90.4" S and -36°60'49.6" W, 247 m a.s.l., between March and April 2021. The experiment was installed in a randomized block design, 5 x 3 x 3 factorial scheme, with five ('Veneranda', 'Camila', 'Elba', 'Vitória Verdinha' and 'Diva'), three commercial substrates for vegetable (Vida Verde<sup>®</sup>, Bioplant 434<sup>®</sup> and Bioplant 401<sup>®</sup>) and three sludge levels (0, 20 and 40 of WTP sludge) and three blocks (one block received shade in the late afternoon. The other two, full sun during the day). The plot consisted of nine plants, five of which used as useful area (Lúcio et al., 2016).

Soil samples at depth of 0-20 cm were collected and sent to the laboratory for chemical analysis, with the following results: pH (water), 7.3; P (Mehlich), 120 mg dm<sup>-3</sup>; K and Na (Mehlich), Ca, Mg and Al (1N KCl), H (calcium acetate pH 7.0), 0.32, 0.87, 3.6, 3.0, 0.0, 0, 3 cmol<sub>c</sub> dm<sup>-3</sup>, respectively. The total organic matter content was

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1.37%. Fe, Cu, Zn and Mn (Mehlich) contents were 120.8, 0.45, 21.24 and 57.77 mg dm<sup>-3</sup>, respectively. The fertilizer used consisted of 80 kg of cattle manure + 60 kg of goat manure + 10 kg of the 16-00-20 NPK formula + 10 kg of castor bean cake (*Ricinus communis* L.), using 30 g plant<sup>-1</sup> in the foundation fertilization, according to Cavalcanti (2008).

Beds had 1.0 m in width, with spacing of 0.30 x 0.30 m between plants and between rows. Soil preparation, from turning over and homogenizing, was manually performed. Fifteen days after planting, manual weeding was performed. The irrigation system, installed on site, was in the microsprinkler mode. However, it was not used due to the rainfall regularity during the experimental period, totaling 94 mm. During the experimental period (35 days), average, minimum and maximum temperatures were 21.7 and 29.8°C, respectively.

All plants in the useful area of the plot were quantified, obtaining the stand, weighing shoots (excluding damaged, diseased or defective leaves), with the aid of digital scale (kg) and the quantified yield (stand x weight; Mg ha<sup>-1</sup>).

Data obtained were arranged in a double-entry M matrix of g x e dimension, with 'g' genotypes and 'e' environments, in which each of the nine environments was obtained from the combination of substrates and WTP sludge levels. In the data analysis, the mixed linear model ( $y = X\beta + Zu + \epsilon$ ) was adopted, where: 'y' is a vector of response variable 'y';  $\beta$  is a vector of unknown fixed effects  $\beta$ ; 'u' is a vector of random effects 'u'; 'X' is a design matrix relating 'y' to  $\beta$ ; 'Z' is an n × m design matrix

relating 'y' to 'u'; and ' $\epsilon$ ' is a vector of random errors ' $\epsilon$ '. The deviance analysis (Anadev) was carried out, in which effects were evaluated by the LRT test (likelihoodratio test) at 5% probability by the X<sup>2</sup> test. Both, genotype and environments were assumed to be random (Model 3), using the argument 'random == "all"' of the function waasb(). The variance components were estimated by function "vcomp".

In the stability analysis, the WAASB index, described by Olivoto et al. (2019), and the ASV index (AMMI stability value), described by Purchase et al. (2000), were adopted, in which the lower the index, the more stable the genotype. Considering that the WAASB method is based on the decomposition of singular values (DSV), applied to the GE interaction matrix, obtained by a linear-mixed effect model, it was possible to obtain the biplots Y x WAASB and AMMI2 (PC1 x PC2). Cultivar means were predicted by the BLUP method. All analyses were performed in the R software (R Core Team, 2021), from the metan package, version 1.16.0 (Olivoto et al., 2020).

## **Results and Discussion**

Significant interaction (P<0.01) was observed between cultivars and growing environments, both for the seedling quality index (SQI) and for the commercial lettuce yield (Table 1), demonstrating the differential performance of cultivars in the different substrate compositions. Therefore, the use of methods that seek to increase efficiency in recommending the cultivars that best interact with the substrate composition is justified.

 Table 1. Deviance analysis (Anadev) and variance components (VC; %) for the seedling quality index (SQI) and commercial yield of five lettuce cultivars and nine environments.

| Effect            | SQI      |        |        | Yield    |         |        |
|-------------------|----------|--------|--------|----------|---------|--------|
|                   | Deviance | LRT    | VC (%) | Deviance | LRT     | VC (%) |
| Cultivar (Cult)   | 183.6    | 1.38ns | 10.1   | 463.2    | 5.24**  | 11.9   |
| Environment (Env) | 175.4    | 1.79** |        | 432.6    | 1.58ns  | 12.7   |
| Cult x Env        | 145.1    | 7.84** | 67.6   | 446.2    | 15.17** | 22.4   |
| Block/Env         |          |        |        |          |         | 20.3   |
| Residual          |          |        | 22.3   |          |         | 32.7   |

\*\* and ns: significant at 1% and not significant at 5% by the  $X^2$  test.

It was observed that the genetic factor contributed with 10.1 and 11.9% of the phenotypic variation, for the SQI and yield, respectively (Table 1). On the other hand, the G x E interaction and the residual variance, respectively, were the most important variations, which most contributed to the phenotypic variation, evidencing the influence of the substrate compositions on the cultivars. Meier et al. (2021) started that greater residual variation is associated to the components of the random effects that are considered in the prediction of the response variable.

The SQI ranged from 0.19 for 'Veneranda' cultivar, indicating less developed seedlings, to 0.29 for 'Diva' cultivar, more developed and above average (Table 2). Both indexes (WAASB and ASV) indicated the 'Camila' cultivar as the most stable, reflecting predictable behavior in all environments (Souza et al., 2020). According to Oliveira et al. (2016), stable genotypes contribute little to

GE interaction. On the other hand, the 'Diva' cultivar had the highest values, being the most unstable.

| Cultivar           | Stability indexes |       |       |                              |       |       |  |  |  |
|--------------------|-------------------|-------|-------|------------------------------|-------|-------|--|--|--|
|                    | SQI               |       |       | Yield (Mg ha <sup>-1</sup> ) |       |       |  |  |  |
|                    | Mean              | ASV   | WAASB | Mean                         | ASV   | WAASB |  |  |  |
| 'Veneranda'        | 0.19              | 0.748 | 0.185 | 5.26                         | 1.891 | 0.425 |  |  |  |
| 'Camila'           | 0.22              | 0.267 | 0.078 | 4.50                         | 1.347 | 0.360 |  |  |  |
| 'Elba'             | 0.22              | 1.197 | 0.235 | 5.41                         | 1.431 | 0.578 |  |  |  |
| 'Vitória Verdinha' | 0.21              | 0.327 | 0.095 | 5.76                         | 0.192 | 0.501 |  |  |  |
| 'Diva'             | 0.29              | 1.724 | 0.320 | 6.04                         | 0.992 | 0.971 |  |  |  |
| Mean               | 0.23              | 0.852 | 0.183 | 5.39                         | 1.171 | 0.567 |  |  |  |

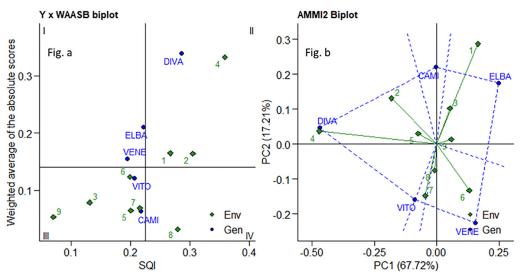
Table 2. Mean and stability indices of five lettuce cultivars for the seedling quality index (SQI) and for yield.

When taken to the field, the commercial yield ranged from 4.50 to 6.04 Mg ha<sup>-1</sup> for 'Camila' and 'Diva' cultivars, respectively. 'Vitória Verdinha' and 'Veneranda' cultivars were classified as stable and unstable, respectively, using the ASV method. By the WAASB index, 'Camila' and 'Diva', respectively. The latter is considered more accurate, as it considers all significant PCs in the AMMI analysis, different from the former, which considers only PC1 and PC2.

Considering that the WAASB method uses the multiplicative effects of the principal component analysis to unfold the GE interaction, it was observed, for SQI and yield, that the first two accumulated 85.9 and 84.1% of the total variation, respectively. This information indicates low existence of uncontrolled factors (noise), increasing

the quality of estimates.

The composition of substrates 1, 2 and 8 promoted higher SQI than the general average (Figure 2a). This biplot allows visualizing the stability and adaptability, in which the 'Diva' cultivar had the highest SQI when specifically associated with substrate 4 (quadrant II), specific adaptability (Souza et al., 2020); however, it was the most unstable (long vector and large angle in relation to the abscissa), according to Yan et al. (2007). As it is a heat tolerant cultivar, mechanisms responsible for higher energy expenditure promote greater phosphorus demand, so the higher P concentration in this substrate (52%) can explain its performance. Both, 'Diva' cultivar and environment 4 (Bioplant 434 + 0% sludge), were those that most contributed to GE interaction (Figure 2a).



**Figure 2.** Biplot Y x WAASB (a) and AMMI2 (b) for the predicted seedling quality index (SQI) of five lettuce cultivars in nine environments. Substrate + WTP sludge ratio: 1) 'Vida Verde' + 0% sludge; 2) 'Vida Verde' + 20% sludge; 3) 'Vida Verde' + 4 0% sludge; 4) Bioplant 434 + 0% sludge; 5) Bioplant 434 + 20% sludge; 6) Bioplant 434 + 40% sludge; 7) Bioplant 401 + 0% sludge; 8) Bioplant 401 + 20% sludge; 9) Bioplant 401 + 50% sludge. VENE: 'Veneranda'; CAMI: 'Camila'; VITO: 'Vitória Verdinha'.

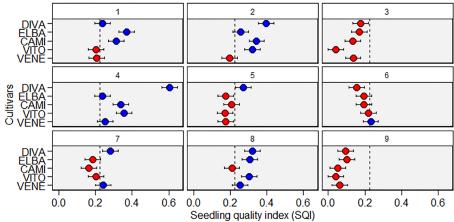
Cultivars that are close to the origin, 'Veneranda' and 'Elba', tend to show general adaptation (Figure 2a) which, according to Duarte & Vencovsky (1999), tend to have sub-optimal performance, which is confirmed in this research (Table 2). Environments 5 and 7, with short vectors, close to the origin (low scores), provide little information about the genotype and should not be used to discriminate the best cultivars (Yan et al., 2007).

Environment 9 promoted lowest SQI values, probably due to its physical composition, consisting of 80.1% of fine particles and 13.1% of organic matter. According to Ludwig et al. (2014), substrates that present a large percentage of small particles, when associated with small containers, promote less aeration, due to greater microporosity, with reflections on poor drainage and seedling development.

The effects of the GE interaction, from PC1 and PC2 (Figure 2b), showed the relationship of each cultivar with the specific substrate, as well as the formation of five mega environments, such as substrates 2, 4, 5, and 1, 3, 9, which were grouped together, being, therefore, similar to each other. Environment 9 (Bioplant 401 + 40% sludge), close to the origin, promoted low growth conditions for

all cultivars. The negative effect of the 40% sludge dose can be observed in the negative correlation between substrates 4 and 6 which, being the same input (Bioplant 434), promoted unfavorable conditions for the growth of the 'Diva' cultivar.

In general, environment 4 (Bioplant 434 + 0% sludge) promoted the best conditions for all cultivars, reflecting in SQI above the average, highlighting the 'Diva' cultivar, not only in this environment, but also in 2, 3, 5, 7 and 8 environments; 'Elba', in environments 1 and 9; and 'Veneranda', in environment 6 (Figure 3). On the other hand, environment 9 (Bioplant 401 + 40% sludge) promoted the lowest rates, followed by environments 2, 5 and 6.



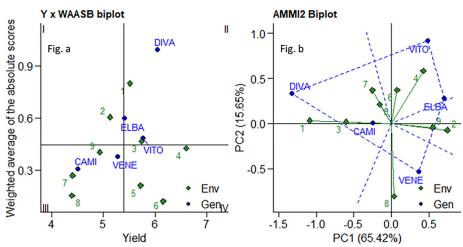
**Figure 3.** Predicted seedling quality index of five lettuce cultivars in each environment. Blue and red indicate cultivars that had BLUPs above and below the average, respectively. Horizontal error bar represents 95% confidence interval by the t test. Substrate + WTP sludge ratio: 1) 'Vida Verde' + 0% sludge; 2) 'Vida Verde' + 20% sludge; 3) 'Vida Verde' + 40% sludge; 4) Bioplant 434 + 0% sludge; 5) Bioplant 434 + 20% sludge; 6) Bioplant 434 + 40% sludge; 7) Bioplant 401 + 0% sludge; 8) Bioplant 401 + 20% sludge; 9) Bioplant 401 + 50% sludge. VENE: 'Veneranda'; CAMI: 'Camila'; VITO: 'Vitória Verdinha'.

'Diva' and 'Vitória Verdinha' cultivars had above average yield (Figure 4a). However, unlike the SQI, at field level, the 'Vida Verde' substrate (0 and 40% sludge) promoted the most favorable environment for the development of the 'Diva' (specific adaptation) cultivar seedlings, probably due to the contribution of super simple, potassium nitrate and NPK (14-16-18), present in this substrate. Likewise, 'Vitória Verdinha' showed specific adaptation to substrate 4 (Bioplant 434 +0% sludge).

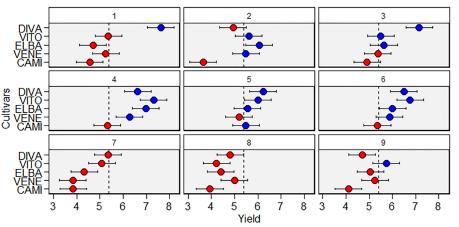
Environments 5 and 6 (Bioplant 434 + 20 and 40% sludge) had the smallest WAASB index (quadrant IV), and were considered those that promote more stability, with yields close to the average, with little contribution to GE interaction (Oliveira et al., 2016). The 1 x 2 and 1 x 9 substrates showed negative correlation, indicating an antagonistic effect on cultivars; 4 x 6, positive, indicating beneficial effect, mainly on the 'Vitória Verdinha' cultivar

(Figure 3b). Environments 1 and 2, with long vectors and large angles to the horizontal axis, enabled identifying unstable genotypes (Yan et al., 2007), such as 'Diva' and 'Elba' cultivars (Table 2).

In general, the Bioplant 434 substrate with up to 40% sludge promoted above-average predicted yield (BLUP), while the opposite occurred for the Bioplant 401 substrate. 'Vida Verde' presented intermediate performance in relation to the sludge doses. 'Camila' cultivar presented below average performance in all environments, probably due to the semiarid climate of the region.



**Figure 4.** Biplot Y x WAASB (a) and AMMI2 (b) for yield of five lettuce cultivars in nine environments. Substrate + WTP sludge ratio: 1) 'Vida Verde' + 0% sludge; 2) 'Vida Verde' + 20% sludge; 3) 'Vida Verde' + 40% sludge; 4) Bioplant 434 + 0% sludge; 5) Bioplant 434 + 20% sludge; 6) Bioplant 434 + 40% sludge; 7) Bioplant 401 + 0% sludge; 8) Bioplant 401 + 20% sludge; 9) Bioplant 401 + 50% sludge. VENE: 'Veneranda'; CAMI: 'Camila'; VITO: 'Vitória Verdinha'.



**Figure 5.** Predicted commercial yield of five lettuce cultivars in each environment. Blue and red indicate cultivars with BLUPs above and below average, respectively. Horizontal error bar represents 95% confidence interval by the t test. Environments (Substrate + WTP sludge ratio): 1) 'Vida Verde' + 0% sludge; 2); 'Vida Verde' + 20% sludge; 3) 'Vida Verde' + 40% sludge; 4) Bioplant 434 + 0% sludge; 5) Bioplant 434 + 20% sludge; 6) Bioplant 434 + 40% sludge; 7) Bioplant 401 + 0% sludge; 8) Bioplant 401 + 20% sludge; 9) Bioplant 401 + 50% sludge. VENE: 'Veneranda'; CAMI: 'Camila'; VITO: 'Vitória Verdinha'.

In the initial evaluation, in the seedling stage, the addition of 40% of WTP sludge reduced the characters linked to the seedling quality index. However, at field level, it was observed that seedlings resumed vegetative growth, probably due to the residual effect of nutrients present in substrates. The high organic matter content present in the WTP sludge, associated with the fine particle content, may have increased water retention in the rhizosphere, reflecting better development and productivity, as observed by Nagase & Dunnett (2011).

#### Conclusions

There is no universal commercial substrate that can be used for the production of lettuce seedlings.

'Vida Verde' (0 and 20% sludge), Bioplant 434 (0% sludge) and Bioplant 401 (20% sludge) substrates promote better seedling quality index, highlighting the 'Diva' cultivar.

The commercial yield of lettuce was higher using Bioplant 434 and 'Vida Verde' with up to 40% of sludge, highlighting 'Diva' and 'Vitória Verdinha' cultivars.

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## References

Associação Brasileira de Normas Técnicas. ABNT. 2004. NBR 10.004: resíduos sólidos – classificação. ABNT, Rio de Janeiro, Brasil. 77 p.

Aboughadareh, A.P., Abad, H.N.C., Mehrvar, M.R., Asadi, A., Amini, A. 2021. MGIDI and WAASB indices: The useful approaches for selection of salt-tolerant barley genotype at the early growth and maturity stages. *Research Square* 1: 1-36.

Brito, S.A., Silva, M.J., Pereira, A.R. 2021. Utilização do lodo da ETA de Xique-Xique, BA: produção de mudas de *Cnidoscolus quercifolius* originária da Caatinga. *Revista Sertão Sustentável* 3: 1-13.

Brunini, O., Lisbão, R.S., Bernardi, J.B., Fornasier, J.B., Pedro Júnior, M.J. 1976. Temperatura-base para alface cultivar "white boston", em um sistema de unidades térmicas. Bragantia 35: 213-219.

Cavalcanti, F.J.A. 2008. Recomendação de adubação para o Estado de Pernambuco: 2ª aproximação. Instituto Agronômico de Pernambuco, Recife, Brasil. 212 p.

Climate-Data. Clima Alagoas. 2022. Available at: https:// pt.climate-data.org/america-do-sul/brasil/alagoas-214. <Accessed on 22 Mar. 2022>.

Correa, B.A., Pereira, M.C., Martins, J.S., Ribeiro, R.C., Silva, E.M. 2019. Reaproveitamento de resíduos orgânicos regionais agroindustriais da Amazônia Tocantina como substratos alternativos na produção de mudas de alface. *Revista Brasileira de Agropecuária Sustentável* 9: 97-101.

Cunha, G.D., Stachiw, R., Quadros, K.M. 2020. Lodo de estação de tratamento de água como componente para germinação de mudas florestais. *Revista Ibero Americana de Ciências Ambientais* 11: 40-53.

Dickson, A., Leaf, A.L., Hosner, J.F. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *The Forestry Chronicle* 36:10-13.

Duarte, J.B., Vencovsky, R. 1999. Introdução genótipos x ambientes: uma introdução à análise AMMI. Sociedade Brasileira de Genética, Ribeirão Preto, Brasil. 61 p.

Gonçalves, F., Souza, C.H.U., Tahita, F.S., Fernandes, F., Teixeira, R.S. 2017. Incremento de lodo de ETA em barreiras impermeabilizantes de aterro sanitário. *Revista* DAE 65: 5-14.

Gull, A., Lone, A.A., Wani, N.U.I. 2019. Biotic and abiotic stresses in plants. In: Oliveira, A.B. (Ed.). *Biotic and abiotic stresses in plants*. IntechOpen, London, UK. p. 3-8.

Guto, R., Silva, E.S. 2018. Produção de mudas de tomateiro, pimenteiro e pepineiro. In: Brandão Filho, J.U.T.,

Freitas, P.S.L., Berian, L.O.S., Goto, R. (Ed.). Hortaliças-fruto. Eduem, Maringá, Brasil. p. 387-400.

Lúcio, A.D., Santos, D., Cargnelutti Filho, A., Schabarum, D.E. 2016. Método de Papadakis e tamanho de parcela em experimentos com a cultura da alface. *Horticultura Brasileira* 34: 66-73.

Ludwig, F., Guerrero, A.C., Fernandes, D.M. 2014. Caracterização física e química de substratos formulados com casca de pinus e terra de subsolo. *Cultivando o Saber* 7: 152-162.

Meier, C., Marchioro, V.S., Meira, D., Olivoto, T., Klein, L.A. 2021. Genetic parameters and multiple-trait selection in wheat genotypes. *Pesquisa Agropecuária Tropical* 51: e67996.

Nagase, A., Dunnett, N. 2011. The relationship between percentage of organic matter in substrate and plant growth in extensive green roofs. *Landscape and Urban Planning* 103: 230-236.

Nascimento, W.M., Croda, M.D., Lopes, A.C.A. 2012. Produção de sementes, qualidade fisiológica e identificação de genótipos de alface termotolerantes. *Revista Brasileira de Sementes* 34: 510-517.

Nataraj, V.N., Bhartiya, A., Singh, C.P., Devi, H.N., Deshmukh, M.P. 2021. WAASB-based stability analysis and simultaneous selection for grain yield and early maturity in soybean. Agronomy Journal 113: 3089-3099.

Oliveira, R.C., Panno, B.A. 2011. Formação de mudas de repolho em substratos a base de húmus, vermiculita e fertilizantes. *Cultivando o Saber* 4: 105-111.

Oliveira, V.M., Hamawaki, O.T., Nogueira, A.O., Sousa, L.B., Santos, F.M., Hamawaki, R.L. 2016. Selection for wide adaptability and high phenotypic stability of Brazilian soybean genotypes. *Genetics and Molecular Research* 15: gmr.15017843.

Olivoto, T., Lúcio, A.D.C., Silva, J.A.G., Marchioro, V.S., Souza, V.Q., Jost, E. 2019. Mean performance and stability in multi-environment trials I: Combining features of AMMI and BLUP techniques. Agronomy Journal 3: 2949-2960.

Olivoto, T., Lúcio, A.D.C. 2020. metan: An R package for multi-environment trial analysis. *Methods in Ecology and Evolution* 11: 783-789.

Purchase, J.L., Hatting, H., Deventer, C.S.V. 2000. Genotype × environment interaction of winter wheat (*Triticum aestivum L.*) in South Africa: II. Stability analysis of yield performance. South African Journal of Plant and Soil 17: 101-107.

R Core Team. R: A language and environment for statistical computing: R Foundation for Statistical Computing (4.1.1). 2021. Vienna, Austria. Available at: https://www.R-project.org/. <Accessed on 10 Sep. 2021>.

Salvador, J.O., Moreira, A., Marcante, N.C. 2013. Waste use as substrate to yield guava seedlings. *Semina* 34: 2793-2802. Souza, M.H., Pereira Júnior, J.D., Stecklig, S.M., Mencalha, J., Dias, F.S., Rocha, J.R.A. S.C., Carneiro, P.C.S., Carneiro, J.E.S. 2020. Adaptability and stability analyses of plants using random regression models. *Plos One* 15: e0233200.

Teodoro, M.S., Pereira, A.M.L. 2021. Use of fish waste in the production of organic compounds for lettuce seedling production. *Engenharia Sanitária e Ambiental* 26: 441-449.

Verma, A., Singh, G. 2021. Stability, adaptability analysis of wheat genotypes by AMMI with BLUP for restricted irrigated multi location trials in Peninsular zone of India. *Agricultural Sciences* 12: 198-212.

Vries, I.M. 1977. Origin and domestication of Lactuca sativa L. Genetic Resources and Crop Evolution 44: 156-174.

Wei, S., Zhang, L., Huo, G., Ge, G., Luo, L., Yang, Q., Yang, X. 2021. Comparative transcriptomics and metabolomics analyses provide insights into thermal resistance in lettuce (Lactuca sativa L.). Scientia Horticulturae 289: 110423.

Yan, W., Kang, M.S., Ma, B., Woods, S., Cornelius, P.L. 2007. GGE Biplot vs. AMMI analysis of genotype-by-environment data. *Crop Science* 47: 643-653.

Zhou, J., Wang, J.Z., Hang, T., Li, P.P. 2020. Photosynthetic characteristics and growth performance of lettuce (*Lactuca sativa* L.) under different light/dark cycles in mini plant factories. *Photosynthetica* 58: 740-747.

Zuffo, A.M., Aguilera, J.G., Lima, R.E., Alves, C.Z. 2020. Substrates for the production of lettuce seedlings. *European Journal of Horticultural Science* 85: 372-379.

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