

## Potassium dose and nutritional diagnosis of virus-free garlic

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### Abstract

Potassium (K) fertilizer application in garlic (*Allium sativum* L) is commonly based on current cultivars that are normally infected with phytopathogenic viruses. Garlic are being developed using meristem culture, but methods for K diagnosis in this type of garlic need to be validated. The objective was to evaluate methods for diagnosing nutritional status of virus-free garlic as a result of K doses through use of a specific K+ meter in soil solution and foliar sap, and to determine foliar K content and its relationship with yield and quality of bulbs. Treatments consisted of K<sub>2</sub>O doses: 0, 50, 100, 250 or 500 kg ha<sup>-1</sup> established in 5 separate locations. In all locations, K<sub>2</sub>O was applied in a single dose, before planting and incorporated in the 0-0.2 m layer. Response of garlic to K fertilization was low, with marketable yield being significant at a single location, with the highest marketable yield associated with 309 kg ha<sup>-1</sup> of K<sub>2</sub>O. The K content of the soil solution and the K in the foliar sap increased with application of increasing K dose. Due to the low response of marketable yield of garlic to K fertilization, technologies of nutritional diagnosis of K was not efficient. Therefore, future studies should focus on different soil K concentrations to validate the sensibility of these technologies.

**Keywords:** *Allium sativum*, plant nutrition, potassium in foliar sap, potassium in soil solution, soil solution

### Introduction

Fertilizer recommendations for garlic production systems, particularly for potassium (K), have commonly been determined using cultivars infested with phytopathogenic viruses (CQFS-RS/SC, 2004). Advanced techniques, such as meristem cultivation that increases vegetative vigor, reduces disease pressure, maximizes yield, and improves bulb quality, are considered as the best management practice to maximize yield in garlic (Santos et al., 2017).

The interaction of soil organic matter and soil texture in association with chemical and climatic conditions, as they influence K dynamics, have not been investigated for virus-free garlic. Recommendations for K fertilization are required for these garlic, since application of K fertilizer is essential to ensure yield and quality (Metwally et al., 2024; Wang et al., 2024).

Nutrient analysis in tissue samples help growers

monitor, evaluate, and adjust K fertilizer rates with low cost and high efficiency, but sometimes with delays in receiving results (Arrobas et al., 2018). Alternatively, use of real-time diagnosis of plant, or soil, samples is needed to determine fertilizer K rate application. Evaluation of K level in foliar sap and soil solution are rapid tests providing information on nutritional state of plants and soil nutrient availability, which would allow a more precise decision on the adequate dose of fertilizer to use during crop development (Bityutskii et al., 2017). Real-time diagnostic methods for soil, or plant nutritional, K status have not been evaluated for decision making in garlic derived from meristem culture. Strategies were evaluated for K fertilizer application and alternative technologies of nutritional diagnosis in virus-free garlic for yield and bulb quality.

The aim of the current study was to evaluate methods for diagnosing nutritional status of virus-free

garlic as a result of K doses through use of a specific K+ meter in soil solution and foliar sap, and to determine foliar K content and its relationship with yield and quality of bulbs.

### Materials and Methods

The same experimental protocol was used in collaboration with growers in 5 locations in the mid-eastern part of Santa Catarina, Brazil, during 2015 and 2016 growing seasons. Soils in the areas are classified as Nitossolo Bruno Distrófico with a clay texture, according to Brazilian Soil Classification System (Embrapa, 2018), which correspond to the Typic Hapludox (Soil Survey Staff, 2022). According to the official recommendation of lime and fertilization for southern Brazil (CQFS-RS/SC, 2004), the available K contents are "high" for location 1 and "medium" for locations 2 to 5. The climate is classified as oceanic or Cfb, temperate and constantly humid with a mild summer. Physical and chemical attributes of the soils of the 5 areas varied (**Table 1**).

During the season, a porous capsule extractor was installed in each plot at a 20 cm depth and soil solutions collected in the differentiation phase of plants (125 days after planting - DAP). Extracted solutions were stored in expanded polystyrene boxes and kept on ice. Soil K content was measured using a LaquaTwin B-743 (Horiba Ltd., Kyoto, Japan).

Samples of foliar sap were obtained at the same time as soil solution samples. Tissue samples consisted of 10 leaves (4th youngest fully expanded leaf) collected from random plants in each plot. The foliar sap (0.1 mL) was collected from a 1.5 cm piece from the basal part of each leaf using a garlic press, stored in expanded polystyrene boxes, and kept on ice. Foliar sap K content was measured using a LaquaTwin B-743 (Horiba). The 10 leaves were washed, dried in a forced air circulation oven at 65±5°C to constant mass, ground, and subjected to K analysis according to Tedesco et al. (1995).

Garlic bulbs were harvested from the 1 m center

**Table 1.** Physical and chemical attributes of soils used in the experiments with K doses in 2015 and 2016 growing seasons.

Growing season	Location	Coordinates	Clay <sup>a</sup> g·kg <sup>-1</sup>	OM %	pH H <sub>2</sub> O	P — K		Ca	Mg	CEC <sub>pH 7.0</sub>
						Mehlich <sup>-1</sup> --- mg·dm <sup>-3</sup> ---	---			
2015	1	26.981696 S 50.825729 W	55	3.9	6.2	10.5	248.0	9.3	1.7	13.18
	2	27.048185 S 50.782615 W	62	4.3	5.7	7.4	86.6	6.9	2.0	16.56
	3	27.160526 S 50.798687 W	63	3.9	5.8	9.9	89.2	6.1	2.6	13.94
2016	4	26.981812 S 50.825656 W	55	4.9	6.1	3.1	76.0	9.2	3.6	18.50
	5	27.005582 S 50.847597 W	58	3.7	5.8	21.8	88.0	8.5	2.0	15.48

<sup>a</sup> Clay by pipette method; OM (organic matter) by Walkley-Black method; pH in water (v/v); P and K by extractor Mehlich-1; Ca, Mg, and Al by extractor 1 mol·L<sup>-1</sup> KCl; CEC = cation-exchange capacity.

Virus-free garlic bulbils, obtained by meristem culture, of cv. Chonan, was used in all locations. Bulbils were manually planted during the first half of June in both years at 45 bulbils m<sup>-2</sup>. All garlic bulbils were pre-treated during 04 hours in a solution with abamectin (200 mL 100 L<sup>-1</sup> water). Beds were prepared with a rotary tiller, were 5 m in length and comprised of 6 rows with 27 cm between rows and 10 cm between plants. A 1 m skip was used to separate plots. Treatments were established in a randomized complete block design (r = 4) with the K<sub>2</sub>O rate treatments: no K<sub>2</sub>O (0), or 50, 100, 250 or 500 kg ha<sup>-1</sup>. Fertilizer was applied as potassium chloride (60% K<sub>2</sub>O) on the soil surface and incorporated into a 0-20 cm deep layer with a rotary tiller before garlic was planted. Nitrogen and phosphorus fertilizers were applied according to established methods (CQFS-RS/SC, 2004) to obtain 15 ton yield of commercial bulbs and established crop management practices related to irrigation and pest management followed Epagri (2002).

of each plot at 140 DAP in 2015 and 148 DAP in 2016. They were weighed after 40 days of natural drying. Marketable bulbs corresponded to the sum of #2 (< 32 mm ø), #3 (32–37 mm ø), #4 (37–42 mm ø), #5 (42–47 mm ø), # 6 (47–56 mm ø) and #7 (> 56 mm ø) bulbs, which were classified according to Luengo (2018). Bulbs with secondary growth and damage were considered culls. Total yield was the sum of marketable and cull yield. At harvest, soil sampling was performed in the 0-20 cm layer to determine available K following Tedesco et al. (1995).

Data were subjected to analysis of variance for each location in each year using the R-studio (ver. 3.03, R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>). When the F value of the ANOVA was significant for the main effect of fertilizer K rate, a polynomial regression was performed. Pearson's correlation test was conducted among all response variables to evaluate their potential use as tools for diagnosis of K in garlic.

The most economical dose of potassium chloride was calculated following Zelelew et al. (2016), equaling the derivative of the second-degree equation by the equivalence relation: price of the kilo of potassium chloride applied/price of the kilo of garlic sold, equal  $US\$0.34/US\$2.57 = 0.1323$  for 2015 and  $US\$ 0.37/US\$ 2.14 = 0.11729$  for 2016.

## Results and Discussion

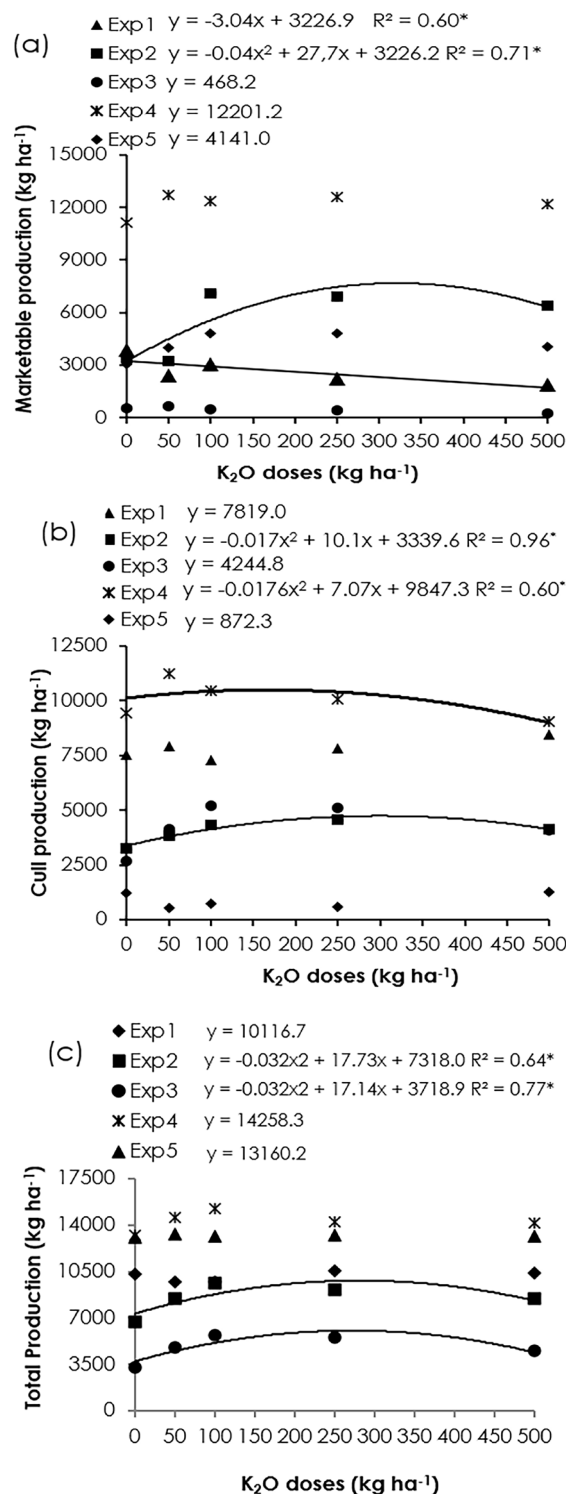
Dose of K affected marketable production at locations 1 and 2 (Figure 1a), in locations 2 and 4 for cull production (Figure 1b), and in location 3 for total production (Figure 1c). Compared with the average in the state of Santa Catarina for total production of 7.5 and 10.4 t ha<sup>-1</sup> in 2015 and 2016, respectively (Conab, 2018), yield of marketable garlic was low in locations 1, 3 and 5, average in location 2, and high in location 4. The negative response in marketable production, and higher cull production in 2015 may be explained by unfavorable climatic conditions. There were late cold temperatures occurring on 13 and 14 September (-2.5°C, both days), coinciding with the differentiation phase of garlic, and high rainfall from the middle, to the end of the production cycle, October to December (Figure 2). This favored false-branching, or super-sprouting, in addition to a high incidence of bacterial infection (Wu et al., 2016).

In location 1, there was a linear decrease in marketable yield with increase of K; in location 2 the highest marketable yield was associated with a dose of 309 kg ha<sup>-1</sup> of K<sub>2</sub>O (Figure 1a). This positive result was verified by increased marketable yield, even though K levels in the soil were above critical levels (CQFS-RS/SC, 2004) (Table 1). Garlic plants may have absorbed non-exchangeable K forms (Vieira et al., 2016), hence the lack of response in commercial yield in 4 of the 5 experimental locations. The data also suggest the need to review the critical K levels in soil for garlic, which is included in the group with the highest K demand by CQFS-RS/SC (2016).

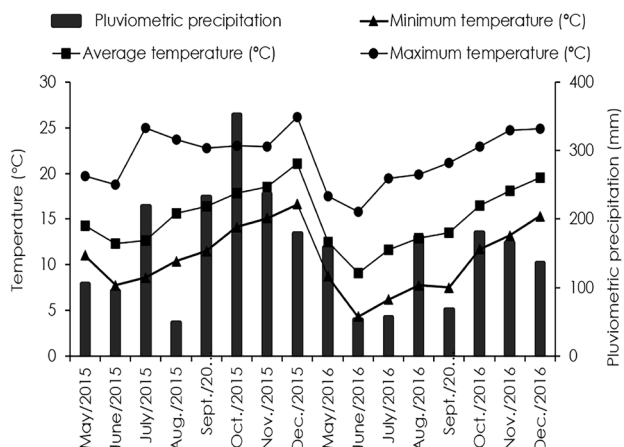
The amount of K that should be applied depends on the level of this nutrient in the soil; where it was found there was no difference in average weight and total yield of bulbs in response to K doses (Wang et al., 2002). This can be attributed to the level in the soil being sufficient for normal plant development, as well as satisfactory bulb yield. The same authors reported that for each ton of garlic produced, 9.1 to 10.1 kg of K<sub>2</sub>O was absorbed by the crop. The amount of K to be applied should be between 60 and 150 kg ha<sup>-1</sup> of K<sub>2</sub>O, depending on the level of K in the soil, the yield to be reached and the soil characteristics (Wang et al., 2002).

Production of cull garlic was influenced by K

dose in locations 2 and 4, with quadratic increases in production. Maximum production was associated with 298 and 287 kg ha<sup>-1</sup> of K<sub>2</sub>O, respectively (Figure 1b). Total garlic production was influenced by application of K only in location 3; maximum yield was associated with application of 271 kg ha<sup>-1</sup> of K<sub>2</sub>O (Figure 1c).



**Figure 1.** Marketable (a), cull (b), and total production (c) of virus-free garlic as a function of application of K doses in 5 locations; \*, \*\* significant at 5 or 1% probability.



**Figure 2.** Precipitation, maximum, minimum and average monthly temperatures during the period experiments.

The K dose influenced similar numbers of nutrient diagnosis technologies for soil solution in 2015 and 2016. The effect of treatment on soil K content occurred at locations 1, 3 and 5, for K content in soil solution, with significance differences in locations 3-5. The K in the foliar sap was different in locations 3-5, and the foliar K content only in location 5 (Table 2). Sensitivity of the diagnosis technologies was not different.

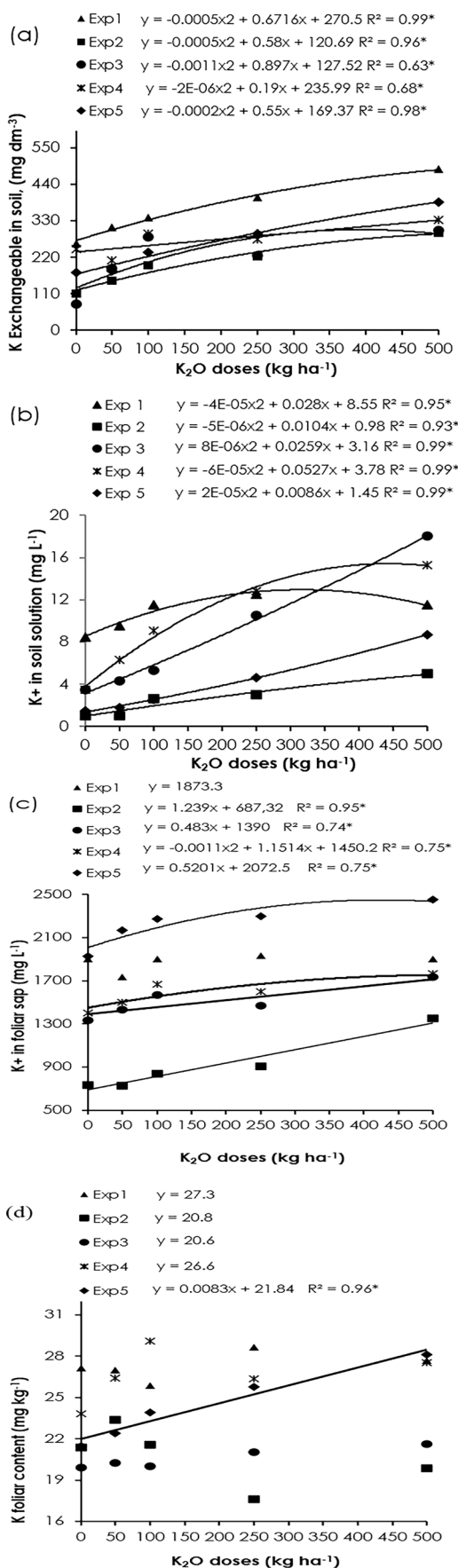
**Table 2.** Analysis of variance response of K dose on K levels available in: soil (KS), soil solution (K SS), foliar sap (K FS), and in leaves (K CF) of virus-free garlic in 5 Locations.

Location	Technologies of nutritional diagnosis			
	KS	K SS	K FS	K CF
1	64.6**	1.7ns	1.2ns	0.4ns
2	38.4ns	38.2ns	25.9ns	1.7ns
3	7.8**	71.5**	6.0*	3.1ns
4	3.8ns	378.3**	3.7**	2.8ns
5	52.1**	93.1**	5.2**	4.4*

ns, \*\*, not significant or significant at 5 or 1% probability by F test, p < 0.05.

\*see table 1 for coordinates of each location

Exchangeable K in the soil in all locations increased quadratic as the K dose increased (Figure 3a). The K content of the soil solution increased with K dose, with a quadratic increase in contents in all locations (Figure 3b). The K applied in the soil at planting linearly increased contents in foliar sap in locations 2, 3 and 5 (Figure 3c) and quadratic increased in contents in location 4. The foliar K content exhibited a linear effect only in location 5 (Figure 3d) with increase of K dose. A specific amount of K in foliar sap presents a greater potential of use for nutritional diagnosis than analysis of foliar K. Potassium is the most abundant cation in plant tissues; however, it is not part of any organic structure or molecule, being found as a free or adsorbed cation, which makes it easily interchangeable from cells or tissues, with high intracellular mobility (Sardans & Peñuelas, 2021).

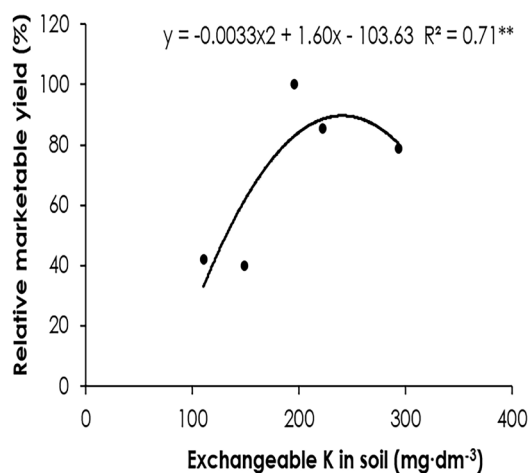


**Figure 3.** Exchangeable K in: soil (a), soil solution (b), foliar sap (c), and foliar K content (d) as a function of application of K dose applied at planting in 5 locations; \*\*, \*\* significant at 5 or 1%, probability.

This explains the higher concentration of K in foliar sap. The relationship between K fertilization and K contents in foliar sap is not well documented compared to N fertilization and its effects on nutrient parameters associated with the plant (Hahn et al., 2020).

The response of virus-free garlic to K fertilization was low and only in location 2 was there a significant increase in marketable yield due to increase of K applied to the soil. Levels of K in the soil were sufficient for development of plants and yield of the bulbs. Because location 1 indicated a linear decrease with application of K dose, and the quadratic behavior in marketable production is associated only with location 2 with the maximum marketable production of garlic bulbs associated with 207 kg ha<sup>-1</sup> of K<sub>2</sub>O (Figure 1a), critical levels for the diagnostic technologies were established only from location 2 which is likely 242.4 mg dm<sup>-3</sup> of K (Figure 4) and this concentration is classified as highly available (CQFS-RS/SC, 2004) in the value of CTC of location 2 (136-270 mg dm<sup>-3</sup> K), which would indicate application of 350 kg ha<sup>-1</sup> of K<sub>2</sub>O, i.e., 47% more than necessary to reach the same range of content considered adequate, obtained in the present location.

There was low efficiency of diagnostic technologies of K associated with marketable yield



**Figure 4.** Relationship between critical levels of exchangeable K in soil and relative marketable yield of virus-free garlic bulbs harvested in location 2 of 2015 crop. \*\* significant at 1% probability.

**Table 3.** Economic dose of potassium chloride, as a function of marketable and cull garlic production and fertilizer costs, 2015 and 2016 growing seasons.

Location/ Crop classification	Economic dose kg·ha <sup>-1</sup>	Increase of production	Fertilizer cost (US\$) ---- kg·ha <sup>-1</sup> of garlic ----	Increase of yield	Relative production <sup>a</sup>
2 / Marketable	307.9	4603.1	44.5	4558.6	100%
4 / Cull	271.0	476.5	50.6	425.9	100%

<sup>a</sup> Percent of bulb production obtained with the most economical dose in relation to maximum production

of garlic. For location 2, the only one with an increase of marketable yield as a function of K fertilization, no adjustment of the relative marketable production with contents of K<sup>+</sup> was obtained, K content in the foliar sap and foliar K content. It is necessary to conduct studies in soils with low concentrations of K, since soils of the region where the experiments were conducted, due to their natural conditions, have naturally high to very high K levels. Given the low occurrence of soils in South Brazil, these technologies will have low potential of use.

Studies that evaluated the economic viability of fertilizer and corrective use (Natale et al., 2011), indicate the most economical dose of the input; such methodology applies to calibration experiments, like the one here, to better guide decisions of each crop. Using regression equations (Figures 1, 2), and current prices of production and inputs, the most economical dose of potassium chloride for production areas can be calculated. The economic dose calculated for location 2 was 308.0 kg ha<sup>-1</sup> of potassium chloride. The expected revenue, due to fertilizer application, can be determined by increase in garlic production (4,603 kg ha<sup>-1</sup>), subtracting cost of fertilizer in kg of garlic, the increase in yield that application of fertilizer provided was 4,559 kg of garlic. The most economical dose for other experiments and/or productions (Table 3), considering production presented the convex quadratic behavior can be calculated. Due to the low response of marketable production of garlic to K fertilization, the technologies used in the nutritional evaluation of K are not efficient.

**Conclusions**

The response of the virus-free garlic culture to K fertilization was low, with marketable production being significant in one of five experiments, with the highest marketable production associated with 309 kg ha<sup>-1</sup> of K<sub>2</sub>O.

The K content of the soil solution and the K in the foliar sap increase with application of increasing K doses.

Due to the low response of marketable production of garlic to K fertilization, the technologies used in the nutritional evaluation of K present low efficiency.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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