Interaction of triacontanol with other plant growth regulators on morphology and yield of field pea (*Pisum sativum* L.)

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ABSTRACT

The effect of a recent plant growth regulator, triacontanol (TRIA), on plant growth and yield of *Pisum sativum* L. was investigated. The experiment was carried out under field conditions at the Instituto Nacional de Innovación Agraria (INIA), La Molina, Lima, Peru, using a completely randomized block design with eight treatments and three replicates. Treatments consisted in the foliar application of TRIA alone and in all possible combinations with three plant growth regulators based on auxins (AUX), gibberellins (GA), and cytokinins (CK), on pea plants cv. Rondo. The highest green pod yields were obtained with the application of TRIA+AUX+GA+CK, and TRIA+AUX+CK. The TRIA+AUX increased the values of the yield variables while TRIA+GA increased the values of the morphological variables. TRIA+CK showed a stimulating effect on morphological variables and number of grains per pod, while TRIA+AUX+CK acted synergistically on yield variables since their combined effect overweighed the effect of each growth regulator separately. Treatments with TRIA exceeded the control treatment in yield variables, indicating its great potential to be used in sustainable agriculture to guarantee food security in the future.

Key words: auxins, cytokinins, gibberellins, plant physiology, legumes.

Introduction

The field pea (*Pisum sativum* L.) belongs to the Fabaceae family and is one of the oldest domesticated species for human and livestock consumption. It is widely cultivated around the world (Wu et al., 2019) and predominant in world trade, representing about 35-40% of the total trade in legumes. It is also among the most consumed vegetables worldwide (Ratnayake et al., 2001). The pea is an important nutritional crop (Liu et al., 2015) since its grains have high contents of protein (18-30%), vitamins and minerals. Additionally, its shoots and leaves can be used for fresh consumption as leafy vegetables (Santos et al., 2014), with a good demand in the national and international markets. Field pea is a profitable agricultural product. However, this profitability is diminished in many regions by various factors such as environmental problems, incorrect agronomic management, and increase in production costs due to the rise in the application of fertilizers and pesticides (Erisman, 2011). Agronomic management is especially critical because long-duration varieties with low yields are still sown in production areas and are susceptible to different diseases and pests (Wu et al., 2019). For this crop, correct physiological and nutritional management is imperative,
with biostimulants and plant hormones playing an important role (Bertolin et al., 2010; Cato et al., 2013; Martínez González et al., 2017).

Plant growth regulators are small molecules that can trigger different physiological processes related to the growth, development, and defense of plants (Pozo et al., 2015). They are also known as phytohormones, but this term is not used frequently in agriculture (Davies, 2010). Plant hormones, growth regulators, and inhibitors have been used in practice to increase yield, improve quality, or alleviate the adverse effects induced by biotic or abiotic stresses (Csukasi et al., 2009). Different classes of hormones have already been characterized, including abscisic acid, auxins, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonates, strigolactones, etc. (Depuydt & Hardtke, 2011). All of them have been linked in one way or another to growth regulation (Santrner et al., 2009; Wolters & Jürgens, 2009). Cytokinins, auxins, gibberellins, and brassinosteroids are considered essential for growth of mutant phenotypes in which hormone biosynthesis or perception is disrupted; cytokinins regulate cell proliferation, while gibberellins promote cell elongation and auxins are involved in both processes. Furthermore, brassinosteroids are essential for cell elongation, but may also play a role in cell division (Nakaya et al., 2002; Hardtke et al., 2007). All these hormones can regulate a high number of processes in a unique and independent way. However, cooperation and interrelationship between signaling metabolic pathways appears to exist, as it follows from the superimposed influence on various cellular processes (Hardtke et al., 2007).

New hormones and plant growth regulators are still being discovered, and their interrelationships, mechanisms of action, and relationships at the metabolic level are being exhaustively studied (Gomez-Roldan et al., 2008; Kuppusamy et al., 2009). Among these substances is triacontanol (TRIA), a growth regulator recently used in commercial applications despite the fact that it was discovered a few decades ago in natural waxes. It can exert stimulating effects, and may also play a role in cell division (Nakaya et al., 2002; Hardtke et al., 2007). All these hormones can regulate a high number of processes in a unique and independent way. However, cooperation and interrelationship between signaling metabolic pathways appears to exist, as it follows from the superimposed influence on various cellular processes (Hardtke et al., 2007).

The experiment was carried out in the experimental fields of the Instituto Nacional de Innovación Agraria (INIA), La Molina, Lima, Peru (12°04’48” S, 76°56’44” W at 243 m a.s.l.) between September and December 2015. To determine the effect of the application of triacontanol (TRIA) and the growth regulators on peas, pea seeds cv. Rondo were used. Rondo is a commercial cultivar with a determinate growth habit, semi-late, with straight pod and rough grain suitable for fresh consumption, and with medium maturation appreciated by farmers for its yield and pod size. Climate characteristics of the field site are described in Table 1. Four pea seeds were sown per hole under drip irrigation at a distance between plants of 0.75 x 0.2 m, with 24 experimental plots of 9 m² and a total area of 320 m² (experimental plots + edge spaces). Average soil characteristics of the field site were: sand, 53%; silt, 28%; clay, 19%; organic matter, 1.12%; P; 17.4 mg kg⁻¹; K, 291 mg kg⁻¹; pH, 7.7, and electrical conductivity, 1.62 dS m⁻¹. The soil analysis was carried out according to the guidelines established by Burt (2014). Subsequently, manual thinning was done, leaving three plants per stroke and a final planting density of 200,000 plants ha⁻¹.

At 43 d after sowing (V4 growth stage), the treatments described in Table 2 were foliar applied. All treatments, except the control, alone or in combination with other plant growth regulators (auxins (AUX), gibberellins (GA) or cytokinins (CK)) contained TRIA, and a foliar-applied potassium fertilizer (K). Other commercial foliar fertilizers from FARMEX S.A. (Peru), such as FX Amino and Powergizer, were also applied to all treatments at the same concentration to replace nitrogen deficiencies.
To evaluate the effect of the treatments on growth and yield, the plant height, number of branches per plant, plant fresh weight, pod length, pod width, number of pods per plant, number of grains per pod and green pod yield (t ha$^{-1}$) were determined at harvest.

A completely randomized block design was used, with eight treatments and three replicates, for a total of 24 experimental units. The results were subjected to a normality test and analysis of variance (ANOVA) by the F test, and the means were compared with the least significant Fisher’s test (LSD) at 5% probability of error, using the statistical software R. Pearson’s correlation test at $P \leq 0.05$ was applied to assess the significance of correlation coefficients.

Table 2: Description of the treatments and doses of the plant growth regulators used in the experiment.

| Name          | Description                                                                 |
|---------------|------------------------------------------------------------------------------|
| TRIA+AUX      | 0.5 mg L$^{-1}$ triacontanol + 10 mg L$^{-1}$ NAA                            |
| TRIA+GA       | 0.5 mg L$^{-1}$ triacontanol + 10 mg L$^{-1}$ GA$_3$                         |
| TRIA+CK       | 0.5 mg L$^{-1}$ triacontanol + 1 mg L$^{-1}$ KIN                           |
| TRIA+AUX+CK   | 0.5 mg L$^{-1}$ triacontanol + 0.5 mg L$^{-1}$ NAA + 1 mg L$^{-1}$ KIN       |
| TRIA+AUX+GA+CK| 0.5 mg L$^{-1}$ triacontanol + 0.5 mg L$^{-1}$ NAA + 1 mg L$^{-1}$ GA$_3$ + 1 mg L$^{-1}$ KIN |
| TRIA+K        | 0.5 mg L$^{-1}$ triacontanol + 3000 mg L$^{-1}$ K$_2$O                     |
| TRIA          | 0.5 mg L$^{-1}$ triacontanol                                                |
| CONTROL       | Treatment without any application of triacontanol or growth regulators      |

NAA - 1-Naphthaleneacetic acid; GA$_3$ - gibberellic acid; KIN - kinetin; K$_2$O - potassium oxide.

Results and discussion

Growth parameters

The plant growth regulators applied in this study caused changes in plant height. The application of TRIA + GA obtained the highest plant height (143.5 cm), and the control obtained the lowest value (79.8 cm), which was 15.6% lower than the average obtained in the experiment (Tab. 3). The height of the pea plant is a characteristic determined by its genetics (Weeden, 2007), and the interaction with growth-promoting phytohormones such as GA (Wang et al., 2017). The control of this experiment showed a height greater than that reported by Anchivilca Rojas (2018) (68 cm), Santos et al. (2018) (60 cm), and Rodriguez Quispe (2015) (52 cm). According to Checa Coral et al. (2020), this could be due to environmental conditions and an efficient supply of resources since the resulting phenotype is the interaction of the genotype and the environment. The positive interaction of TRIA and GA agrees with that proposed by Shukla et al. (1992), who mention that triacontanol potentiates the effect of GA. The opposite is observed with AUX, since TRIA decreases its influence as a "destruction" effect is produced. The intensity of this effect is specific in each cultivar (Henry & Gordon, 1980); auxins at low concentrations inhibit stem growth in favor of root growth and development (Davies, 2010; Taiz et al., 2014). No effects were observed with foliar application of potassium because the soil was rich in this element (291 mg L$^{-1}$) and the application of potassium would keep the plants under conditions of "luxury consumption" (Marschner, 2011).

Regarding the number of branches per plant, there were no statistical differences in the analysis of variance for the treatments. Most of the treatments obtained similar values to those of the control, except for TRIA+GA, with a value 21.8% less than the average, and TRIA+AUX+GA+CK, with a value 23.5% greater than the control and 26.3% greater than the average (Tab. 3). The trend is repeated for pod length and plant fresh weight regarding the highest and lowest values in the treatments. However, for pod width, the control showed the lowest value of 1.66 cm, TRIA+AUX showed an intermediate value of 1.76 cm, and TRIA+AUX+GA+CK obtained the highest value with 1.83 cm.

The number of branches plant (NBP) and the pod width are also genetically determined characteristics, but the former is more susceptible to variations due to environmental conditions and/or agronomic practices than the latter (presence of the n gene, Wehner & Gritton, 1981). Pod width did not exhibit significant or percentage statistical variation from all variables, showing a less marked incremental effect of the negative interaction of TRIA with AUX and CK applied jointly (Fig. 1). On the other hand, for NBP, the treatments...
with CK increased the branching of the shoots (Müller & Leyser, 2011) and the number of branches in the plants (Taiz et al., 2014).

Pearson’s correlation coefficients showed interesting data regarding the relationships between variables. Within the vegetative parameters, plant height was negatively correlated with pod length (-0.271) and the number of branches per plant was positively correlated with pod length (0.442*) and yield (0.731**). The variable pod length was positively correlated with plant fresh weight (0.626**), number of grains per pod (0.823**), and green pod yield (0.654**). A positive correlation was also observed between these last three variables. However, these variables were not positively correlated with pod width (Tab. 4). In contrast with the values obtained in the variable plant height, the average pod length in this experiment (9.9 cm) was lower than that obtained by Anchivilca Rojas (2018) (10 cm), Rondinel Ruiz (2014) (10.2 cm), and Rodríguez Quispe (2015) (11.4 cm). Triacontanol application resulted in an increase of 11.3% in yield over the control (Fig. 2). A positive interaction was also observed with treatments containing CK and, to a lesser extent, with foliar potassium. A slight negative interaction was observed with AUX and a higher negative interaction was registered with gibberellins.

The effect of CK is significant since they promote greater pod setting in legumes (Carlson et al., 1987) and increase the width, length, and weight of pods (Mosjidis et al., 1993). Potassium also has a slight incremental effect on fruiting and a higher pod setting in legumes (Marschner, 2011). However, this is not reflected by a luxury consumption of K. The trend of the effect of the hormonal interaction is repeated on pod length, as well as on plant fresh weight, except that a negative interaction with foliar potassium was also recorded. Although gibberellins favor the vegetative growth of the plant when applied in excess (Taiz et al., 2014), this growth is very fast, forming tall but weak stems with lower dry matter content and fresh weight of the plant. The mixture of the three hormones considerably increases the plant fresh weight due to a better regulation of physiological processes and the internal hormonal balance in the plants (Figs. 1-3).

### Yield parameters

The lowest values of all yield variables (number of pods per plant, number of grains per pod, and green pod yield) were obtained with TRIA+GA. The highest values were recorded with TRIA+AUX+GA+CK, in which all the hormones were used, except in the number of pods per plant, where the best treatment was TRIA+AUX+CK (Tab. 3). The number of pods per plant showed a different behavior between treatments, although it was highly positively correlated with the number of grains per pod (0.485*) and yield (0.654**). A positive correlation was also observed between these last three variables. However, these variables were not positively correlated with pod width (Tab. 4). In contrast with the values obtained in the variable plant height, the average pod length in this experiment (9.9 cm) was lower than that obtained by Anchivilca Rojas (2018) (10 cm), Rondinel Ruiz (2014) (10.2 cm), and Rodríguez Quispe (2015) (11.4 cm). Triacontanol application resulted in an increase of 11.3% in yield over the control (Fig. 2). A positive interaction was also observed with treatments containing CK and, to a lesser extent, with foliar potassium. A slight negative interaction was observed with AUX and a higher negative interaction was registered with gibberellins.

### TABLE 3. Effect of plant growth regulators on growth and yield of pea plants.

| Treatment                  | PH (cm) | NBP | PL (cm) | PW (cm) | NPP | NGP | PFW (g) | Yield (t ha⁻¹) |
|----------------------------|---------|-----|---------|---------|-----|-----|---------|----------------|
| TRIA+AUX                   | 86.5b±8.3 | 2.7a±0.4 | 9.67b±0.7 | 1.77b±0.03 | 9.5b±0.1 | 6.1a±0.7 | 130.2b±21.5 | 7.43c±0.8     |
| TRIA+GA                    | 143.5a±4.6 | 2.2b±0.3 | 8.26a±0.4 | 1.83a±0.09 | 3.7a±0.9 | 4.6a±0.01 | 93.5b±11.5 | 2.15c±0.3     |
| TRIA+CK                    | 95.8a±6.4 | 2.8a±0.2 | 10.67a±0.1 | 1.74a±0.03 | 12.2a±3.4 | 7.9a±0.3 | 143.3a±15.6 | 8.47c±0.9     |
| TRIA+AUX+CK                | 85.0a±6.1 | 2.8a±0.2 | 10.29a±0.4 | 1.68a±0.02 | 15.0a±1.5 | 7.7a±0.2 | 154.5a±7.2  | 10.53b±0.9    |
| TRIA+ AUX+GA+CK            | 91.5a±4.5 | 3.5a±0.5 | 10.71a±0.7 | 1.83a±0.06 | 14.7b±3.2 | 2.7a±0.5 | 198±34.9    | 12.15a±1.4    |
| TRIA+K                     | 85.7a±3.3 | 2.7a±0.2 | 10.46a±0.4 | 1.81a±0.03 | 9.0a±1.3 | 7.7a±0.6 | 120.6a±15.4 | 7.30a±1.0     |
| TRIA                       | 88.7a±3.3 | 2.7a±0.2 | 10.21a±0.3 | 1.73a±0.05 | 12.8a±0.9 | 7.2a±0.4 | 135.8a±9.3  | 8.88a±0.4     |
| CONTROL                    | 79.8a±7.2 | 2.8a±0.2 | 9.33a±0.5 | 1.66a±0.04 | 13.3a±1.5 | 5.9a±0.3 | 122.2a±14.6 | 7.98a±1.0     |
| F-Test                     | ns       | ns   | ns      | ns      | *   | **  | ns      | **            |
| Mean                       | 94.56    | 2.77 | 9.95    | 1.75    | 11.27| 6.98 | 137.29   | 8.11         |

TRIA - triacontanol, AUX - auxins, GA - gibberellins, CK - cytokinins, K - foliar-applied potassium, PH - plant height, NBP - number of branches per plant, PL - pod length, PW - pod width, NPP - number of pods per plant, NGP - number of grains per pod, PFW - plant fresh weight. ns - not significant at P>0.05; *: P≤0.05; **: P≤0.01.
TRIA+AUX, TRIA+GA and the control did not exceed the average value obtained in the experiment of seven grains per pod (Tab. 3).

Within the yield components, the number of pods per plant with the application of TRIA decreased by 3.8% compared to the control (Fig. 1A). No positive interactions were observed with the mixture of AUX and CK or with the mixture of the three hormones. However, a negative interaction was recorded with AUX and CK applied separately since the AUX-CK relationship is imbalanced in physiological processes. A large decrease was observed with GA since they favor vegetative growth (Davies, 2010), and foliar potassium application obtained values below the control (32.5% less).

The number of pods per plant is one of the most important yield components in legumes. GA decreased this yield variable because it promotes growth to the detriment of fruiting and formation of plant flowers (Davies, 2010). CK promote pod formation and show synergism with auxins (Cato et al., 2013). The positive impact of CK has been observed in plants, such as Artemisia grown in vitro (Rasool et al., 2013), showing a greater effect with the mixture of the three growth regulators (Cato et al., 2013).

Foliar-applied potassium has a positive effect on the control and an interaction with TRIA, similar to that occurring with CK. Regarding the variable green pod yield, the values obtained ranged between 2.15 and 12.15 t ha⁻¹ with highly significant statistical differences between treatments (P<0.01). TRIA+AUX+CK and TRIA+AUX+GA+CK
exceeded 10 t ha⁻¹, and TRIA+GA showed a decrease of 73.1% relative to the control. TRIA+Aux and TRIA+K did not exceed the control. The application of TRIA alone showed an increase of 11.3% relative to the control (Fig. 2).

Pearson’s correlation coefficients of the yield variables showed that the number of pods per plant was positively correlated with number of grains per pod (0.485*), number of branches per plant (0.655**), plant fresh weight (0.670**), and yield (0.883**), and negatively correlated with plant height (-0.573**). The number of grains per pod was also positively correlated with the other variables (P<0.05). The same was observed for the variable green pod yield, except for pod width, which is not correlated with any variable (Tab. 4).

Green pod yield is the agronomic trait that helps to determine the profitability of the crop. It also determines if the crop is a good alternative for the farmer, who looks for high yields with increasing emphasis on quality (Espinosa & Ligarreto, 2005; Checa Coral et al., 2017). The high yield values obtained are due to the fact that the values of number of branches per plant, number of pods per plant, and number of grains per pod were also higher. These last two variables are important components of the yield and determine the productivity of plants. The lowest values were obtained in the interaction of TRIA with Aux and GA applied separately. This shows the negative effect of GA on crop yield in general since they decrease pod formation favoring vegetative growth and increasing plant height, an unfavorable issue leading to lodging (Davies, 2010; Hedden, 2016; Wang et al., 2017). It also shows the negative effect of Aux applied alone, since they alter the Aux-CK relationship that is key to the growth and development of plant species (Schaller et al., 2015).

TRIA is a natural growth regulator widely studied in various species, with notable effects on the growth and development of rice (Jadhav et al., 2017), Capsicum annum L. (Sahu et al., 2017), willow (Digruber et al., 2018), sweet potato (Rajak et al., 2018), strawberry (Baba et al., 2017), and guava (Singh et al., 2017). Pearson’s correlation coefficients show a highly positive correlation between yield and the number of pods per plant (0.883**), number of branches per plant (0.731**), number of grains per plant (0.723**), plant fresh weight (0.845**), and pod length (0.654**) (Tab. 4).

The differential effect for TRIA in this experiment was greater, especially in the yield components, with an increase in plant height of 11%, a decrease in number of branches per plant and number of pods per plant of 5.9 and 3.8%, respectively, and increases of 9.5% in pod length, 4.2% in pod width, 11.19% in plant fresh weight and 22.6% in number of grains per pod.

### Conclusions

The results of this study showed that TRIA by itself has no remarkable effect on the growth and yield of Pisum sativum; only when TRIA is combined with other plant growth regulators such as AUX, GA and CK are noteworthy effects seen. AUX, GA and CK increased morphological variables with the exception of the number of branches per plant, and pod length in the case of GA. Within the yield components, AUX and CK increased the number of grains per pod and GA decreased all these variables, showing a negative interaction with TRIA. AUX and CK acted synergistically in almost all the variables evaluated, except for the number of branches for which similar values were obtained. In all these variables, the synergistic action of AUX and CK exceeds the effect of each growth regulator separately. The joint application of foliar-applied potassium fertilizer with TRIA showed an unfavorable effect on the yield and plant fresh weight. The highest green pod yields were obtained with the application of TRIA plus AUX, GA and CK (12.15 t ha⁻¹), followed by the application of TRIA plus AUX and CK (10.53 t ha⁻¹). The trihormonal application with TRIA obtained the best results in the variables.

| TABLE 4. Pearson’s correlation coefficients of the variables tested. |
|---------------------------------|
| PH    | NBP    | NPP | PL | PW | PFW | NGP |
| 0.613** | 0.731** | 0.883** | 0.654** | -0.009 | 0.845** | 0.723** |
| 0.731** | 0.731** | 0.670** | 0.626** | 0.267 | 0.654** | 0.723** |
| 0.655** | 0.731** | 0.873** | 0.654** | -0.009 | 0.845** | 0.723** |
| 0.573** | 0.443* | 0.642* | 0.396 | 0.131 | 0.670** | 0.573** |
| 0.443* | 0.642* | 0.396 | 0.131 | 0.670** | 0.573** | 0.573** |
| 0.225 | 0.243 | -0.232 | 0.131 | 0.626** | 0.267 | 0.670** |
| 0.255 | 0.891** | 0.670** | 0.626** | 0.267 | 0.670** | 0.573** |
| -0.447* | 0.456* | 0.485* | 0.823** | 0.058 | 0.573** | 0.573** |
| -0.573** | -0.271 | ---- | ---- | ---- | ---- | ---- |
| PH - plant height; NBP - number of branches per plant; NPP - number of pods per plant; PL - pod length; PW - pod width; PFW - plant fresh weight, and NGP - number of grains per pod.
because the hormonal relationship is maintained and is not altered by the imbalance of the others.

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Conflict of interest statement
The authors declare that there is no conflict of interest regarding the publication of this article.

Author’s contributions
AHJ obtained the financial support and the experiment site for the project. AHJ and HCS designed the methodology. HCS conducted the research process, performed the experiment, and analyzed the data. HCS wrote the draft and AHJ carried out the revision of the manuscript.

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