EFFECT OF GA_3, KNO_3, AND REMOVING OF BASAL POINT OF SEEDS ON GERMINATION OF SWEET GRANADILLA (*Passiflora ligularis* JUSS) AND YELLOW PASSION FRUIT (*Passiflora edulis* F. *flavicarpa*)[1]

JULIÁN CÁRDENAS[2], CARLOS CARRANZA[2], DIEGO MIRANDA[3], STANISLAV MAGNITSKIY[3]

ABSTRACT - *Passiflora* seeds germinate erratically presenting difficulties for their handling in a greenhouse. The effect of removing of basal point of seeds (RB) and pre-imbibition of seeds of sweet granadilla and yellow passion fruit in 50, 100, 200, and 400 mg mL^{-1} solutions of gibberellic acid (GA_3) or 0.1% KNO_3 solution was studied. The experiment was conducted in greenhouses in La Plata, Colombia. Two accessions PrJ1 and PrJ2 of sweet granadilla were evaluated. There were calculated the final percentage of germination (PG), mean germination time (MGT), and the mean germination rate (MGR). The leaf area and dry mass of seedlings were measured 22 days after sowing (das); with this data, specific leaf area and relation root/shoot were calculated. In all cases, the highest germination percentages were achieved treating seeds with KNO_3 (89, 92, and 87% for yellow passion fruit, PrJ2, and PrJ1, respectively), but the increase in MGR (3.3 germinated seeds per day) and the decrease in MGT (16 days) were only significant for PrJ1. RB had a significant reduction of PG in all cases (28, 12, and 33% for passion fruit, PrJ2 and PrJ1, respectively). With the increase in the concentration of GA_3, PG was reduced for two accessions of sweet granadilla, for yellow passion fruit this trend was not clear, no treatment with GA_3 showed significant differences with the control. Leaf area (24.07 cm2) and dry mass of seedlings (135 mg) were significantly higher than seeds previously treated with KNO_3 only for PrJ1.The solution of KNO_3 0,1% is recommended to improve the germination and initial growth of granadilla seedlings.
Index terms: Pre-imbibition, final percentage of germination, mean germination time, mean germination rate, leaf area.

EFEITO DO GA_3, KNO_3 E O DESPONTE BASAL NAS SEMENTES EM GERMINAÇÃO DE GRANADILLA (*Passiflora ligularis* JUSS) E O MARACUJÁ COMUM (*Passiflora edulis* F. *flavicarpa*)

RESUMO- A germinação desordenada das sementes das passifloras dificulta seu manejo em estufas. Com este trabalho, procuraron-se estudar o efeito do desponte basal (DB) e a pré-embebição das sementes de granadilla (*Passiflora ligularis* Juss) e o maracujá comum (*Passiflora edulis* f. *flavicarpa*) submetidos a quatro concentrações de ácido giberélico (GA_3): 50; 100; 200 e 400 mg mL^{-1}, uma solução de KNO_3 a 0,1% e água como testemunha. O experimento foi realizado em estufas localizadas em La Plata (Colômbia). Para o caso do granadilla, os acessos PrJ1 e PrJ2 foram testados, avaliando os seguintes parâmetros: a) porcentagem da germinação final (PG); b) tempo médio de germinação (TMG); c) velocidade média de germinação (VMG); d) área foliar e massa seca das plântulas 22 dias após a semeadura. Com estes dados, a área foliar específica e a relação raiz/parte aérea foram calculadas. Em todos os tratamentos, as melhores percentagens da germinação foram alcançadas com solução de KNO_3 a 0,1% (89; 92 e 87% para o maracujá comum, PrJ1 e PrJ2, respectivamente). Somente foram significativos o aumento da VMG (3,3 sementes germinadas por dia) e a redução do TMG (16 dias) em PrJ1. O DB gerou uma redução significativa da PG em todos os tratamentos (28; 12 e 33% para maracujá comum, PrJ1 e PrJ2, respectivamente). O aumento na concentração de GA_3 promoveu reduções da PG para os dois acessos de granadilla. Para o maracujá comum, a tendência não foi clara. Nenhum tratamento com GA_3 mostrou diferenças significativas em relação à testemunha. A área foliar (24,07 cm^2) e a matéria seca das plântulas (135 mg) foram significativamente maiores com KNO_3 para o acesso PrJ1. A solução de KNO3 0,1% é recomendada para melhorar a germinação e o crescimento inicial de mudas de Granadilla.
Termos para indexação: pré-embebição, porcentagem da germinação final, tempo médio de germinação, velocidade média de germinação, área foliar.

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[2]Ingeniero Agrónomo, M.Sc. Fisiología de Cultivos, Universidad Nacional de Cultivos, Sede Bogotá. E-mail: julianentero@gmail.com; cecarranzag@gmail.com.
[3]Profesor Asociado, Facultad de Agronomía, Universidad Nacional de Colombia, Sede Bogotá. E-mail: dmirandal@unal.edu.co; svmagnitskiy@unal.edu.co.

INTRODUCTION

The cultivation of sweet granadilla (*P. ligularis*) represents an important item in Colombian export market. Within the market for exotic fruits in 2008 this fruit ranked the second one ($ 2,500,000) (PROEXPORT, 2009). This culture despite being relatively new, has undergone rapid expansion due to favorable soil and climatic conditions for cultivation in the country and its high market demand. In case of yellow passion fruit, Colombia stands out as the third largest producer of this fruit, with 91,870 t produced in 2009 and an average annual rate of 5.6% of production.

The germination of seeds of *Passiflora* is slow possibly due to the exogenous dormancy, which could be a combination of mechanical and chemical dormancy, the last one due to the presence of inhibitors in seeds (DELANOY et al., 2006). In this regard, studies on germination of *P. ligularis* are scarce. Germination in seeds of yellow passion fruit has already been addressed in a number of studies; however, for the environmental conditions of the Colombian fruit producing regions, no studies have been conducted.

Soaking seeds of *P. edulis* Sims (DELANOY et al., 2006) and *P. alata* (ROSSETTO et al., 2000) in solutions of giberrelic acid (GA_3) after performing mechanical scarification favored germination percentage (PG). In seeds of *P. nitida*, soaking in GA_3 increased PG and changed the germination response to light conditions (PASSOS et al., 2004). In *P. giberti* GA_3 application did not overcome the seed dormancy (FERREIRA et al., 2002).Previous experiments in our lab have shown that the response in germination of seeds of *P. ligularis* to the application of GA_3 depends on the temperature and, especially, light conditions, achieving better responses with high concentrations of GA in light and low concentrations in darkness.

The functions specific to gibberellin induction of seed germination include promotion of elongation of hypocotyl and stem (RICHARDS et al., 2001). GA_3-deficient tomato mutants could not initiate the process of seed germination (LIU et al., 1994).

Several nitrogen compounds promote the breaking of seed dormancy and stimulate seed germination, among these KNO_3 is recommended for testing the germination of many species (BRAZIL, 2009) and is mainly related to seed germination of photoblastic seeds, such as of *Passiflora* (BALAGUERA et al., 2010).

The goal of this research was to study the effect of exogenous application of GA_3, mechanical scarification and KNO_3 on the germination of seeds of yellow passion fruit and sweet granadilla under controlled conditions of light and temperature.

MATERIALS AND METHODS

The fruits of two accessions of sweet granadilla named PrJ1 and PrJ2 were collected in the municipality of Palestina, Huila province (Colombia) in a farm located at 1,807 m.a.s.l. with coordinates 01 º 41.630 ‘N and 076 º 07.387’ W. Yellow passion fruit seeds were extracted from fruits obtained from the municipality La Plata, Huila province (Colombia), located in a commercial crop culture at 1,112 m.a.s.l. with coordinates 2 ° 21.830 ‘N and 075 ° 54.908’ W. In all cases, the aryl was removed from seeds by letting the seeds fermenting with the aryl for three days and removing it with a strainer. Seeds without aryl were allowed drying up for three days at room temperature (20 °C) in the darkness. Dried seeds reached humidity contents between 10 and 12%.

In Crop Physiology Laboratory of the Faculty of Agronomy, National University of Colombia, Bogotá campus, the best concentration of KNO_3 was determined for treating the seeds of sweet granadilla, than these were placed to germinate in Petri dishes on filter paper in germination chambers Sanyo LMR-351 (Japan), at 30/20 °C (12 h/12 h), 85% RH in complete darkness. The treatments consisted of applying solutions of 0 (distilled water), 0.1, 0.2, or 0.5% (w / v) KNO_3 throughout the germination test which lasted 32 days.

In a preliminary experiment (data not shown) with sweet granadilla seeds germinated on filter paper, the treatments with best results in the germination of this species were: GA_3 (50, 100, 200 and 400 mg L^{-1}) and removing of basal point of seeds (RB). These treatments, along with the optimal concentration of KNO_3, selected from the preliminary experiment, were tested in greenhouse conditions on two accessions of sweet granadilla and one accession of yellow passion fruit.

In the greenhouse facilities of Corporation CEPASS-Huila located in the municipality of La Plata, the seeds of sweet granadilla and yellow passion fruit were imbibed for 48 hours in solutions of GA_3 (50, 100, 200 and 400 mg L^{-1}), or KNO_3 (0.1% w / v) and distilled water using seeds with removing of basal point and uncutted seeds as controls. The germinated seeds were placed in trays of 50 alveoli filled with Base Substrate Klasmann® (Klasmann-Deilmann, Geeste, Germany) without nutrients added. The trays were watered to field capacity and placed in the dark room (temperature between 18 and

34 °C, relative humidity between 61 and 99%) of the greenhouse for 10 days. Then the trays were moved to the greenhouse plastic cover (temperature between 20 and 33 °C), where they remained for 22 days. The trial lasted a total of 32 days, where seedling emergence was recorded every 2 days.

At the end of both tests, the laboratory and the greenhouse, the percentage of final germination (PG), the average time of germination (ATG) and the mean germination rate (MGR) were calculated

$$TMG = \sum_{i=1}^{k} n_i t_i / \sum_{i=1}^{k} ni \quad (1)$$

$$VMG = \sum_{i=1}^{k} n_{i/} t_i \quad (2)$$

where: n_i = number of germinated seeds in the i^{th} data collection, t_i = time (in days) of the i^{th} data collection and K = time (in days) of the duration of germination test (RANAL and SANTANA, 2006, RASHID et al., 2010).

In the greenhouse, in seedlings of sweet granadilla of both accessions were measured leaf area, total dry weight, dry weight of the stem, leaves and roots of each seedling at 15 days after initiating the emergency, from KNO_3 treatments and controls, taking five replicates per treatment. With these data, the specific leaf area (leaf area / leaf weight) and the ratio root / shoot were calculated.

An analysis of variance (ANOVA) and Tukey's test (P <0.05) were done to determine differences among the treatments using the SAS software V. 9.2.

RESULTS AND DISCUSSION

For the treatment with KNO_3, the highly significant differences among PG were recorded for seed PrJ1 accession; the highest PG, 96.33% was achieved with 0.1% KNO_3. For PrJ2, PG reached 0.1% KNO_3 (88.33%) and did not present statistical differences with the highest PG (92.67%) recorded for that accession. Thus, 0.1% KNO_3 was the concentration that was further selected for testing germination of sweet granadilla seeds (Table 1). This indicates the need to use this salt in germination tests, as well as substrates enriched with potassium nitrate in the commercial propagation of sweet granadilla.

The MGT for both accessions was lower in treatment with KNO_3 (16.09 d to PrJ1 and 19.6 d to PrJ2) (Table 2), this treatment also reached the highest MGR (3.3 d^{-1} seeds PrJ1 and 2.2 d^{-1} for seeds PrJ2), while the lowest values of this index were recorded in the RB treatment (Table 2).

There was higher variability among the treatments in MGT than MGR values (Table 1), indicating that differences in PG were due more to differences in the number of days it takes for seeds to germinate than in the number of seeds germinated per day. This could be related to a more rapid activation of metabolic processes.

Under greenhouse conditions, RB treatment had the highest decrease of PG compared to other treatments (Table 2), and this occurred in seeds of two accessions of sweet granadilla and seeds of yellow passion fruit. In this respect, there was a high presence of fungi near the basal region at the end of the test, suggesting higher sensitivity of this treatment to environmental conditions without full management of external biological agents.

In seeds of sweet granadilla, the response of PG varied among accessions, although both the ANOVA indicated a significant effect (P <0.01) treatments, the only response PrJ1 different from control was in the treatment of removing of basal point of seeds. The other treatments showed increases KNO_3 (92%) compared to the control (82%), but these were not significant (P> 0.05). In the seeds of both accessions, there was a tendency to decrease PG with increasing concentration of GA_3 (Table 2), more marked in PrJ2 which recorded a significant decrease in PG in solution of 400 mg L^{-1} GA_3 compared with other GA_3 treatments, with the control (77.3%) and the treatment of KNO_3 (86.6%).

The content of endogenous nitrate in each seed is dependent on various aspects of genetics and nutrition of the mother plant during the seed formation. It is demonstrated that increasing the amount of nitrate supply to the mother plant during seed maturation lead to less number of dormant seeds (MATAKADIS et al., 2009). In this context, it is likely that there were differences in the nitrate content of seeds among the accessions of sweet granadilla, a factor that could affect the responses to the application of KNO_3, GA_3, and scarification.

Nitrogen compounds affect germination through the detection of nitrogen in the soil. These could stimulate the pentose phosphate pathway in seeds and, thus, seed germination by increasing the oxidation of NADPH to NADH. Nitrate can alter hormone levels by inducing the expression of enzymes that catalyze the inactivation of abscisic acid ABA (CYP707A2) and the biosynthesis of gibberellins (GAox1) (FINCH et al., 2007). In seeds of yellow passion fruit, as well as in seeds of PrJ1, only the RB treatment showed a significant change of the PG compared with the control (88.66%), with a drastic reduction of this value (28%) and reduction in MGR (1.19 germinated seeds per day) (Table 2).

Importantly, the values of germination in controls and those of MGR and MGT in all treatments were better and more homogeneous in seeds of yellow passion fruit than in two accessions of sweet granadilla (Table 2). This may indicate, first, the possibility that the conditions under which the experiment was developed would have been better for the germination of seeds of yellow passion fruit than for the seeds of sweet granadilla. On the other hand, it is likely that the seeds of yellow passion fruit did not exhibit dormancy, it was a weaker dormancy or related with other factors than in seeds of sweet granadilla, which could be related to a longer time of domestication that had the culture of yellow passion fruit with respect to sweet granadilla.

In seedlings of PrJ1, pre-imbibition of seeds in KNO_3 had a significant effect on leaf area, dry weight of leaves, specific leaf area and total plant weight, but its effect was not significant on dry mass of stem and root, or the root / shoot ratio. In case of seedlings PrJ2, no variable showed significant response to seed treatment with KNO_3. Despite this, shoots and roots of PrJ2 showed the tendency to gain more dry weight with seeds soaked in KNO_3 compared to unsoaked seeds (Table 3).

The total mass of the seedling and leaves and leaf area were significantly higher in seedlings coming from seeds PrJ1 treated with KNO_3, keeping the same trend in the case of PrJ2 (Table 3). The opposite occurred with specific leaf area (Table 3), indicating that KNO_3 may contribute more to the expansion of leaves than to the mass gain by leaves.

The observed effect of potassium nitrate is well known in other species and could be explained because it supplies the system requirements of phytochrome in photoblastic seeds (MATAKIADIS et al., 2009), suggesting physiological dormancy mechanisms in seeds of sweet granadilla. In yellow passion fruit the results do not show differences between treatments (data not shown) which could suggest its seeds are less sensible to light stimuli than granadilla seeds.

Due to the environmental dependence of phytochrome functioning, it is likely that the response of germination has been strongly influenced by environmental conditions prevailing in the greenhouse. Additionally, it is important to note that the response of seeds to GA_3 and KNO_3 treatments is strongly influenced by the time of seed storage (PADUA et al., 2011). In the present experiment, seeds were not stored, so this results could change in stored seeds due the natural process of ageing.

TABLE 1- Effect of four concentrations of potassium nitrate on the germination of two accessions of sweet granadilla.

Accession	Concentration of KNO_3 (%)	PG (%)		MGT(d)		MGR (germinated seeds/d)	
PrJ1	0	62.7	c	11.62	b	6.45	bc
	0.1	96.33	a	11.20	b	10.56	a
	0.2	80.33	b	12.98	ab	8.53	ab
	0.5	81.67	ab	14.35	a	6.11	c
PrJ2	0	68.00	b	11.9	b	6.80	a
	0.1	88.33	a	12.51	b	8.53	a
	0.2	86.33	a	15.41	a	6.88	a
	0.5	92.67	a	12.36	b	8.83	a
	CV (%)	10.38		6.03		8.26	

Treatment means in the same column between the same accession followed by the same letter are not significantly different at 1% level of Tukey Test

TABLE 2- Percentage of germination (PG), mean germination time (MGT) and mean germination rate (MGR) of seeds of sweet granadilla for accessions PrJ2, PrJ1 and yellow passion fruit, grown in peat substrate in greenhouse conditions in La Plata (Colombia)

	PG (%)			MGR(germinated seeds/day)			MGT (day)		
	PrJ1	**PrJ2**	**Yellow passion fruit**	**PrJ1**	**PrJ2**	**Yellow passion fruit**	**PrJ1**	**PrJ2**	**Yellow passion fruit**
50GA_3	82.67 a	82.00 ab	88.66 a	2.17 b	2.01 a	3.4 ab	20.81 ab	20.45 ab	13.64 ab
100GA_3	86.00 a	82.67 ab	84 a	2.15 b	1.93 a	2.7 c	21.83 ab	20.83 a	15.82 a
200GA_3	83.33 a	70.67 ab	78.66 a	2.15 b	1.75 a	3.27 bc	21.37 ab	20.65 ab	13.16 ab
400GA_3	76.67 a	65.33 b	82 a	1.97 b	1.78 a	3.46 ab	19.66 a	21.16 b	13.06 b
KNO_3	92.00 a	86.67 a	88.66 a	3.30 a	2.29 a	3.6 ab	19.68 c	16.10 b	13.21 ab
RB	12.00 b	33.33 c	28 b	0.36 c	0.87 b	1.19 d	19.94 bc	18.50 ab	13.8 ab
Control	82.00 a	77.33 ab	88.66 a	2.49 b	1.96 a	3.94 a	20.23 ab	19.34 ab	12.19 b
CV(%)	8.54	10.62	6,61	10.96	12.31	6,62	4.73	3.33	7,21

Treatment means in the same column followed by the same letter are not significantly different at 1% level of Tukey Test

TABLE 3- Total dry mass, dry mass of leaves, leaf area and specific leaf area of seedlings of the accessions and PrJ1 and PrJ2, from seeds soaked in KNO_3 solution or water.

Accession	**Treatment**	**Leaf area (cm^2)**	**Dry mass of leaves (mg)**	**Total dry mass (mg)**	**Specific leaf area (cm^2 mg^{-1})**
PrJ1	Control	13.42 b	35.66 b	68.67 b	0.37 a
	KNO_3	24.07 a	83 a	135 a	0.28 b
PrJ2	Control	14.32 b	44.66 b	79.67 b	0.31 ab
	KNO_3	16.27 ab	50.66 b	90.67 ab	0.32 ab
CV(%)		20.15	14.92	23.55	8.63

Treatment means in the same column followed by the same letter are not significantly different at 1% level of Tukey Test

CONCLUSIONS

The application of KNO_3 to seeds increased and significantly accelerated the germination of fresh seeds of sweet granadilla in laboratory conditions (filter paper) and greenhouse (peat substrate), however, its effect depended on the accession of the seeds. The application of KNO_3 (0.1%) had no significant effect on the germination of yellow passion fruit in greenhouse conditions in the municipality of La Plata. The application of GA_3 (50, 100, 200, or 400 mg L-1) did not significantly increase germination of sweet granadilla seeds or yellow passion fruit seeds in greenhouse conditions in the municipality La Plata. Soaking the seeds in a solution of KNO_3 (0.1%) significantly affected the growth of seedlings in one of the two accessions of sweet granadilla.

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REFERENCES

AGRONET. Análisis-Estadísticas: producción nacional por producto. Available in: Access in: 02 de septiembre de 2011

BALAGUERA, H.E.; ÁLVAREZ G.; CÁRDENAS, J. Efecto de la estratificación fría y la cobertura plástica en semillas de gulupa (*Passiflora edulis* Sims) para la obtención de plántulas. **Revista Actualidad y Divulgación Científica**, Bogotá, v.13, p. 89-97, 2010.

BRASIL. Ministerio da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Brasília: CLAV:DNDV:SNAD:MA, 2009. 395p.

DELANOY, M.; VAN DAMME, P.; SCHELDEMAN, X.; BELTRAN, J. Germination of *Passiflora mollissima* (Kunth) L.H.Bailey, *Passiflora tricuspis* Mast. and *Passiflora nov* sp. Seeds. **Scientia Horticulturae**, Amsterdam, v. 110, p. 198-203, 2006.

FERREIRA, G.; DETONI, A.M.; TESSER, S.M.; MALAVASI.,Y.M.M. Avaliação de métodos de extração do arilo e tratamento com ethephon em sementes de *Passiflora gibert* N.E. Brawn pelos testes de germinação e de tetrazólio. **Revista Brasilera de Sementes**, Viçosa, MG, v.24, p.248-253, 2002.

FINCH, W.E.; CADMAN, C.S.; TOOROP, P.E.; LYNN, J.; HILHORST, H. Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and lightshows common quantitative patterns of gene expression directed by environmentally specific sensing. **Plant Journal**, London, v.51, p.60-78, 2007.

LIU, Y.; BERGERVOET, J.H.W.; VOS, C.H.R.; HILHORST, H.W.M; KRAAK, H.L.; KARSSEN Y.; BINO, R.J. Nuclear replication activities during imbibition of abscisic acid- and gibberellin-deficient tomato (*Lycopersicon esculentum* Mill.) seeds. **Planta**, Berkeley, v.194, p.368-373, 1994.

MATAKIADIS, T.; ALBORESI, A.; JIKUMARU, Y.; TATEMATSU, K.; PICHON, O.; RENOU, J.P.; KAMIYA, Y.; NAMBARA, E.; TRUONG, H. The arabidopsis abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. **American Society of Plant Biologists**, Oregan, v. 49 n. 2, p.949-960, 2009.

PÁDUA, J.G.; SCHWINGEL, L.C.;. MUNDIM, R.C.; SALOMÃO, A.N; ROVERIJOSÉ, A.N. Germinação de sementes de *Passiflora setacea* e dormência induzida pelo armazenamento. **Revista Brasileira de Sementes**, Viçosa-MG, v. 33, n.1, p.80-85, 2011.

PASSOS, I.R.S.; MATOS, G.V.C. ; MELETTI, L.M.M.; SCOTT, M.D.S.; BERNACCI, L.C.; VIEIRAS, M.A.R. Utilizacao do ácido giberélico para a quebra de dormência de sementes de *Passiflora nitida* Kunth germinadas *in vitro*. **Revista Brasileira de Fruticultura**, Jaboticabal, v.26, N.2. p.380–381, 2004.

PROEXPORT. **Informe frutas exóticas, mermeladas y frutas deshidratdas**. Colombia, 2009.

RANAL, M.; SANTANA, Y D.G. How and why to measure the germination process? **Revista Brasileira de Botânica**, São Paulo, v.29, n.1, p.1-11, 2006.

RASHID, H.; ASAEDA, T.; UDDIN. n. The Allelopathic potential of kudzu (*Pueraria montana*). **Weed Science**, Laurence, v. 58, p.47-45, 2010.

ROSSETTO, C.A.V.; CONEGLIAN, R.C.C.; NAKAGAWA, J.; SHIMIZU, M.K.; MARIN, V.A. Germinação de sementes de maracujá-doce (*Passiflora alata* Dryand) em função de tratamento pré-germinativos. **Revista Brasileira de Sementes**, Viçosa, MG, v. 22, n. 1, p. 247-252, 2000.