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SEEDLING GROWTH OF IRRIGATED RICE AS A FUNCTION OF SEED TREATMENT WITH GIBBERELLIC ACID

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ABSTRACT - This study aimed to evaluate the early growth stages of irrigated *Oryza sativa* L. (rice) seedlings from cultivars SCS-112, BRS-7 Taim, BR IRGA 410 and IRGA 417, under three different temperatures, i.e. 15, 20 and 25 ºC, as a result of seed inbibition in a gibberellic acid (GA_3) solution at concentrations of 0, 150, 300, 450, 600 and 750 mg L^{-1}. The lengths of the shoot and the primary root of seedlings were recorded at three, 7 and 21 days from sowings on germination paper. Interactions between the main factors were observed for all the response variables. Cultivar BRS-7 Taim shows a better response three days after sowing to inbibition of seeds in GA_3 solution on shoot and primary root lengths. The response to GA_3 in shoot growth of rice seedlings increases as the temperature is raised, which also increase the concentrations of greater growth increment due to the regulator. Root growth is little affected by the GA_3 at 7 and 21 days after sowing. Rice cultivars have different sensibility to exogenous gibberellin concentrations via seed treatment, reflecting upon seedling performance.
Keywords: environmental stress, growth regulators, *Oryza sativa* L., seed vigor.

CRESCIMENTO DE PLÂNTULAS DE ARROZ IRRIGADO EM FUNÇÃO DO TRATAMENTO DE SEMENTES COM ÁCIDO GIBERÉLICO

RESUMO - Este trabalho teve por objetivo avaliar o crescimento inicial de plântulas das cultivares de arroz (*Oryza sativa* L.) irrigado SCS-112, BRS-7 Taim, BR IRGA 410 e IRGA 417, sob as temperaturas de 15, 20 e 25 ºC, em função da embebição das sementes em solução com as concentrações de ácido giberélico (GA_3) de 0, 150, 300, 450, 600 e 750 mg L^{-1}. Avaliou-se o comprimento da parte aérea e raiz primária de plântulas aos três, sete e 21 dias após a semeadura em papel de germinação. Houve interação entre os fatores para todas as variáveis. A cultivar BRS-7 Taim apresenta maior resposta, no comprimento da parte aérea e raiz primária, à embebição das sementes em solução com GA_3, aos três dias após a semeadura. A resposta ao GA_3 no crescimento da parte aérea das plântulas de arroz aumenta ao passo que a temperatura é elevada, onde também se elevam as concentrações de maior incremento ao regulador de crescimento. O crescimento de raiz aos sete e 21 dias após a semeadura é pouco afetado pelo GA_3. Cultivares de arroz possuem sensibilidade diferenciada à variação na concentração de ácido giberélico no tratamento das sementes, o que reflete no desempenho das plântulas.
Palavras-chave: estresse ambiental, regulador de crescimento, *Oryza sativa* L., vigor de sementes.

INTRODUCTION

Rice (*Oryza sativa* L.) breeding, through selection for shorter plants, resulted in lower levels of endogenous growth regulators, among them gibberellins (MARTINS et al., 2012), a relevant group of molecules that control several aspects of plant growth and development (YAMAGUCHI, 2008).

Despite the advantages of growing shorter plants, the reduction of the level of endogenous gibberellin on rice tends to affect adversely seedling vigor (BEVILAQUA et al., 1993), which can reflect in a slow and uneven plant establishment. This phytohormone regulates the synthesis and secretion of hydrolytic enzymes involved in the mobilization of reserves, promoting germination and early plant growth (O'BRIEN et al., 2010; LINKIES; LEUBNER-METZGER, 2012; ZIMMER, 2012). Other roles of gibberellin involve shoot growth (YAMAGUCHI, 2008), also a relevant trait for crop establishment, allowing for a faster seedling emergence. The exogenous application of gibberellic acid can be considered as an alternative to low levels of this growth regulator in seeds.

However, besides the endogenous issues, low air temperature as well as that of the soil under cultivation, which are frequent in many regions of southern Brazil,

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may also affect germination and initial seedling growth, thus compromising rice establishment and turning it a central issue for its cultivation (MERTZ et al., 2009). The optimum temperature range for germination of rice lies between 20 and 35 ºC, while the range for optimum seedling emergence and establishment ranges between 25 and 30 ºC (YOSHIDA, 1981).

It is thought that the use of growth regulators can reduce the impact of adverse environmental conditions on the performance of seeds, specifically in the case of rice establishment at low temperatures, as it was observed that gibberellic acid used as a seed treatment favored initial seedling growth under such adverse conditions (CHEN et al., 2005; ARSEGO et al., 2006). This leads to the possibility of a routine use of this growth regulator on seed treatment, however, it is necessary to check if the response varies among different cultivars due to temperature changes, what are the optimum concentrations in each case and also if any phytotoxic effects are observed. The assessment of differential responses among rice cultivars is justified, since data from Bevilaqua et al. (1993) had already demonstrated differential sensitivity among rice genotypes to GA_3, however, most of those rice genotypes are no longer being cultivated currently.

This study aimed to evaluate the initial seedling growth of four irrigated rice cultivars under different temperatures, whose seeds were treated to different concentrations of gibberellic acid.

MATERIAL AND METHODS

The research took place at the 'Flávio Farias Rocha Laboratory' for Seed Testing, at the Plant Science Department of the College of Agronomy "Eliseu Maciel", Universidade Federal de Pelotas (UFPel), Capão do Leão, RS. The study was laid as a triple factorial, 4 x 6 x 3 design where the factors studied were: rice cultivar, gibberellic acid concentration and temperature.

Rice seeds from cultivars SCS-112, BRS-7 Taim, BR IRGA 410 and IRGA 417 were imbibed in a solution of gibberellic acid (GA_3) at concentrations of 0, 150, 300, 450, 600 and 750 mg L^{-1} of distilled water during 24 hours, and placed in a germination chamber at a temperature of 25 ºC. After this period, the solution of GA_3 was drained, and four replicates of 20 seeds were sown, totaling 80 seeds per experimental unit. The samples consisted of a germitest paper roll moistened at 2.5 times its dry weight (BRASIL, 2009), and maintained at temperatures of 15, 20 and 25 ºC. For the 15 ºC temperature, the rolls were conditioned in plastic bags in order to maintain their moisture and kept vertically in a B.O.D. growth chamber. For the treatments with temperatures of 20 and 25 ºC, the germitest paper rolls were put in seed germination chambers. The experimental design used was entirely randomized, with four replications. At days three, seven and 21 after sowing (DAS) fifteen of the biggest seedlings were selected to record the lengths of shoots and primary roots, using a millimeter ruler.

Experimental data were subjected to analysis of variance and the effects of the treatments and their interactions were analyzed by the F test ($p \leq 0.05$). When the results were significant, polynomial regression analyses were performed for GA_3 concentrations and growth temperatures effects for each cultivar and response variable.

RESULTS AND DISCUSSION

The analysis of variance indicated a significant interaction between the factors GA_3 concentration and cultivar, concentration of the growth regulator and temperature and yet, between temperature and rice cultivar for the seedling shoot length at three days after sowing (DAS) (Figure 1). In general, increasing GA_3 concentrations promoted longer shoots, but reducing the effect for the range of 330 to 550 mg L^{-1}.

Rice cultivars showed variation in their response to the growth regulator (Figure 1A). BRS-7 Taim was the genotype that presented the best response to GA_3 concentration increase, with an increase of 80% on the shoot length at concentrations of around 430 mg L^{-1}, followed by IRGA 417 with a 40% increase at concentrations around 400 mg L^{-1}. Cultivars BR IRGA 410 and SCS-112 presented a small response to variations in GA_3 concentration, with maximum growth increases of around 17% for concentrations of around 330 and 550 mg L^{-1}, respectively.

Regarding the interaction between concentration of GA_3 and temperature (Figure 1B) it was found that additions of the regulator to the solution in which the seeds were submerged provided reduced effect on shoot growth of seedlings when under low temperature (15 ºC). When temperatures ranged from 20 to 25 ºC the response to GA_3 concentration was notorious, with similar response curves expressing maximum growth at concentrations of around 450 mg L^{-1}. However, at 25 ºC the seedling shoot growth was more accentuated. Finally, to interaction between cultivar and growth temperature (Figure 1C), it showed IRGA 417 and BRS-7 Taim as the best adapted to low temperature development, with higher shoot lengths at 15 ºC and higher shoot length rate up to 20 ºC. For the 20 to 25 ºC temperature range cultivars SCS-112 and BR IRGA 410 showed the highest rates for shoot length increase, while IRGA 417 presented the lowest.

Other authors (BEVILAQUA et al., 1993; CHEN et al., 2005; GROHS et al., 2012) also reported differences between irrigated rice cultivars in response to the treatment of seeds with GA_3, during the initial phase of seedling growth. The results obtained by Grohs et al. (2012) suggested that cultivars more tolerant to low temperatures presented an enhanced growth response to GA_3, especially at lower temperatures. This is consistent with the results of this work, since the two cultivars that presented the highest tolerance to low temperatures (BRS-7 Taim and IRGA 417) were the ones that presented a higher response to the increase in the concentrations of the growth regulator. Similar to what was published by these authors, Bevilaqua et al. (1993), pointed out that genotypes with characteristics of higher vigor during early growth, also showed the highest sensibility to the treatment with gibberellic acid. On the other hand, Chen et al. (2005)

found that not all cultivars evaluated responded to the treatment with GA_3 under low temperature.

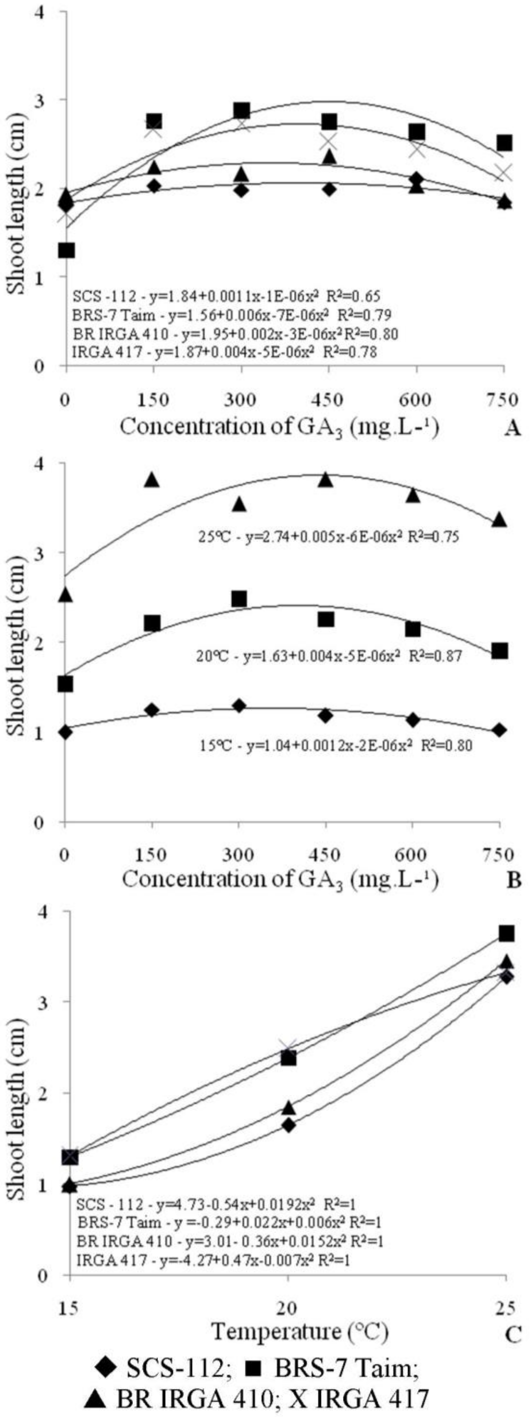

FIGURE 1 - Effect of the concentration of GA_3 (A and B) and temperature (B and C) on seedlings shoot length of irrigated rice cultivars at three days after sowing (DAS).

Based on these data it could be assumed that the response of rice to exogenous GA_3 is dependent on the intrinsic seedling metabolism, being the role of the exogenous growth regulator to enhance a trait already present, at least when considering shoot growth. This suggests that seed treatment with GA_3 does not substitute for an adequate choice of cultivars tolerant to low temperatures and with seeds of high vigor, as well as for sowing in the appropriate dates, but it basically enhances seedling performance much in the same way the actions mentioned are made.

Differences in the cultivar sensibility to GA_3 can also be related to the level of concentration of the endogenous growth regulator within seeds and seedling tissues, as argued by Singh et al. (1981) in a study evaluating the biochemical response to the application of exogenous gibberellic acid on rice. This is consistent with the results obtained by Bevilaqua et al. (1993), who observed that the cultivar that presented the lowest plant height, and probably less content of endogenous gibberellins (MARTINS et al., 2012) responded better to the treatment of seeds with GA_3 for several traits, including shoot length.

In this study we expected a positive response to GA_3 concentrations at three DAS, since this is the closest date to initiation of germination, which is nothing but the resumption of the embryo growth. Upon imbibition, GA_3 stimulates the synthesis and secretion of hydrolytic enzymes such as α-amylase, by the scutellum and aleurone layer, within the starchy endosperm, where they hydrolyze seed reserves, thus making them available for embryo growth (GUERRA, 2004; O'BRIEN et al., 2010; ZIMMER, 2012). The increase on the expression of α-amylase in seedlings due to seed immersion in GA_3 solution has been recorded in rice (VIEIRA et al., 2002).

A triple interaction between GA_3 concentration, cultivar and temperature on seedling growth was recorded at 7 and 21 days after sowing (Figure 2). Again this reinforces that the cultivars exhibited a different response according to the GA_3 concentration, for the temperature range considered in this work. In brief, similar as observed at three DAS, GA_3 enhanced seedling shoot growth, with cultivar and temperature defining the level of gibberellin concentration at which shoot growth peaked.

At 7 DAS, it was reported that as growth temperatures rose so did GA_3 concentrations that yielded the highest seedling growth; consequently, are higher the concentrations at which the effect of GA_3 decreases the increase rate, and the fall in the growth rate becomes less sudden (Figure 2A, 2B and 2C).

At 15 ºC GA_3 concentrations of around 400 mg L^{-1} provided the highest responses for cultivars SCS-112, IRGA 417 and BRS-7 Taim, with diminishing effects at higher concentrations; cultivar BR IRGA 410 maintained a linear growth rate with increasing gibberellin concentrations, differentiating from the others (Figure 2A). Cultivar BRS-7 Taim presented the highest shoot length, regardless of the gibberellin level, which emphasizes its tolerance to low temperatures. At 20 ºC (Figure 2B), cultivars SCS-112, IRGA 417 and BRS-7 Taim showed higher growth efficiency for the interval of GA_3 concentrations between 400 and 600 mg L^{-1}. For BR IRGA 410, the induction to growth continued until the concentration of 750 mg L^{-1}, showing the same growth pattern at 15 ºC, but in general IRGA 417 and BRS-7 Taim presented higher growth values. At 25 ºC (Figure 2C), the concentrations with better response by the different cultivars ranged from 400 to 700 mg L^{-1}. IRGA 417 presented the best increment in response to GA_3, while BRS-7 Taim presented a steady growth increase within the concentration range and BR IRGA 410 and SCS-112 increased their growth rates response to GA_3 only up to around 450 mg L^{-1}, beyond which shoot growth stabilized.

At 21 days after sowing (Figures 2D, 2E and 2F), the cultivars showed a response pattern similar to that at 7 DAS, with differences among them relating to gibberellin concentration and temperature at which shoot growth peaked. Once again, responses to GA_3 became more outstanding as growth temperatures rose and the decline on growth rate due to GA_3 also started at higher concentrations. Besides, as growth temperatures increased all the rice cultivars started to behave in a more similar fashion.

At 15 ºC (Figure 2D), BRS-7 Taim was the cultivar that maintained the highest growth rate in response to GA_3 concentration, until approximately 750 mg L^{-1}, while SCS-112 and IRGA 417 showed high responses up to concentrations of around 450 mg L^{-1} and BR IRGA 410 yielded the lowest growth rate in response to the gibberellin. Cultivars SCS-112 and IRGA 417 stood out by their higher decrease on shoot growth in response to increased GA_3 concentrations. At 20 ºC, SCS-112, IRGA 417 and BRS-7 Taim presented a rise in the shoot length due to the GA_3 until the highest concentration evaluated, where SCS-112 presented the highest values for shoot length whereas BRS-7 Taim and IRGA 417 exhibited a linear response to GA_3 concentrations. This contrasted with the results for BR IRGA 410 which expressed higher growth induction at much lower GA_3 concentrations, close to 450 mg L^{-1}, but rapidly decreasing its response as gibberellin concentration increased. At 25 ºC all cultivars presented a constant increase on shoot growth to higher GA_3 concentrations, with SCS-112 and IRGA 417 exhibiting a linear relationship while for the rest of the cultivars the highest response was achieved at the concentration of approximately 750 mg L^{-1}.

As emphasized, shoot growth response to levels of GA_3 concentration increased as so did the temperature under which seeds initiated germination and seedlings developed, however, with differences between cultivars. BRS-7 Taim, for example, at 21 DAS, was out yielded by the other cultivars as temperatures increased (Figures 2D, 2E and 2F). This agrees with the assumption that the sensibility to GA_3 depends on the seedlings' metabolism, which varies according to the temperature. One of the possible explanations for this response is that enzyme activity is highly dependent on temperature (TAIZ; ZIEGER, 2013) and as already stated, one of the main gibberellin attributions is to regulate the synthesis and activities of enzymes that provide endosperm reserves to the embryo (ZIMMER, 2012).

Even though this enzyme control is dully know, it is not possible to attribute seedling shoot length entirely to exogenous GA_3 have increased the speed of availability of sugars for this growth, because the transduction route for the gibberellin signal pathway in plants is supposedly the same for the induction of both, the α-amylase production and the stem elongation, another outstanding function for this phytohormone (SUN; GUBLER, 2004). Following this concept, the effect could also have been simultaneous, with GA_3 inducing shoot elongation while supplying faster the necessary energy for the latter to occur.

The excessive increase on shoot length due to the GA_3 can impair plant growth, making it prone to lodging or to an imbalance on dry matter partition, affecting tiller formation, as observed by Grohs et al. (2012). The significance of this effect is that the gibberellin concentration that promotes the highest shoot growth may not be the most appropriate for an adequate stand establishment on field.

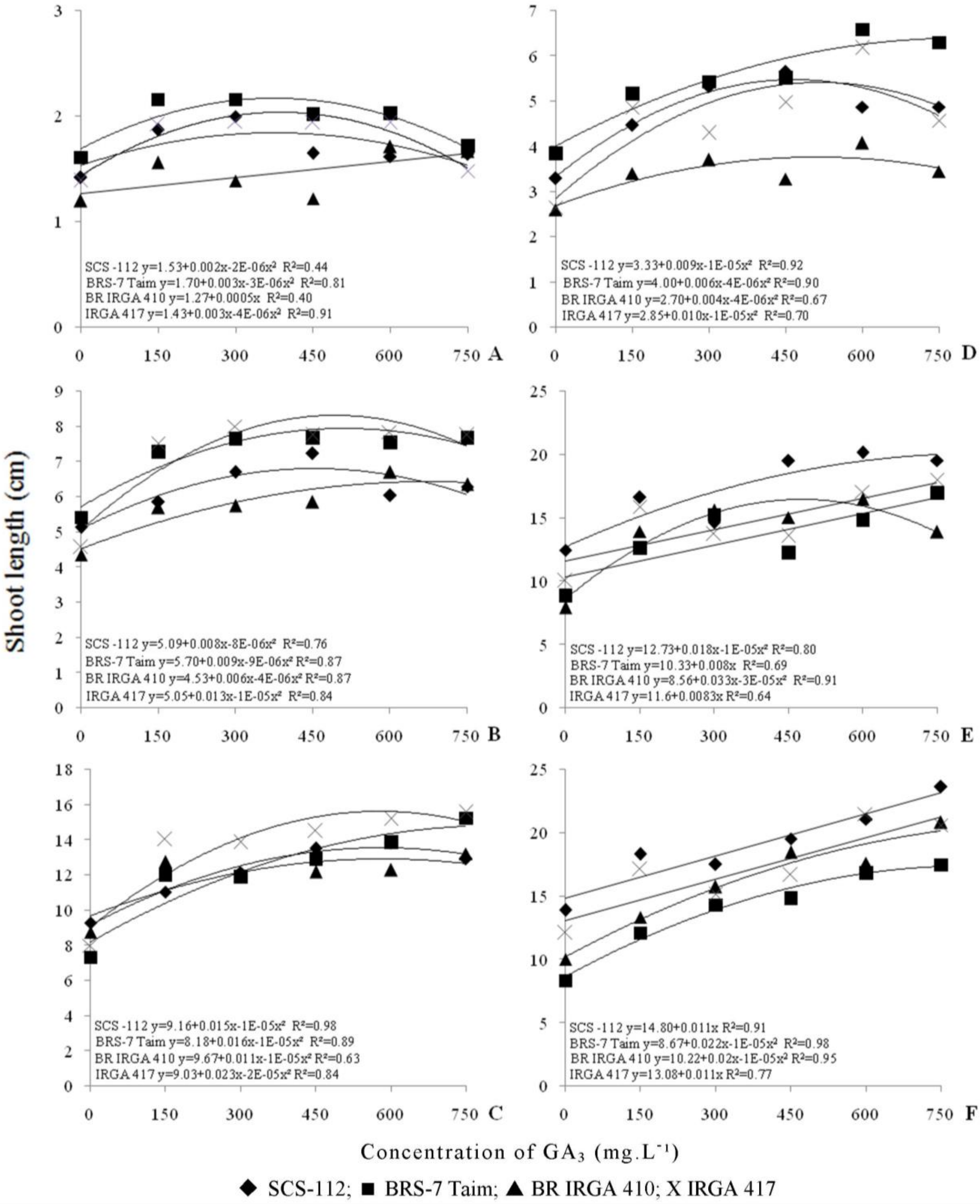

FIGURE 2 - Effect of the concentration of GA_3 on seedlings shoot length of irrigated rice cultivars grown under different temperatures, at 7 and at 21 days after sowing (DAS). 7 DAS: 15 ºC (A); 20 ºC (B); 25 ºC (C); and 21 DAS: 15 ºC (D); 20 ºC (E); 25 ºC (F).

The GA_3 concentrations at which seedling growth rate started to decrease were also mentioned by Bevilaqua et al. (1993), for some traits related to the initial growth of rice seedlings, and also by Albuquerque et al. (2009) in bell pepper, suggesting that the high levels of this exogenous regulator can be phytotoxic or cause a hormonal imbalance. The decrease in the response of shoot growth to GA_3 slowed down at higher temperatures, indicating that when the seedling metabolism is enhanced by temperature they can use or metabolize a higher proportion of this molecule.

The root length presented a different behaviour according to the period in which it was recorded (Figures 3 and 4). At three DAS the interaction between the three factors was observed (Figures 3A, 3B and 3C), however, the performance of the cultivars remained similar under the three levels of temperature. Cultivars SCS-112, BR IRGA 410 and IRGA 417 suffered a severe inhibition on root growth as GA_3 concentrations increased; however, this inhibition decreased as the growth temperature rose. BRS-7 Taim showed a completely distinct behaviour, contrary to the other cultivars, since root growth was highly stimulated by GA_3 up to concentrations of around 500 mg L^{-1}, where at a 15 ºC an increase in excess of 700% on root length was observed, after which further increases on gibberellin concentrations reduced seedling growth. Despite showing a similar response trend across temperatures, the reduction growth rate at high GA_3 concentrations for BRS-7 Taim, was more evident at low temperatures.

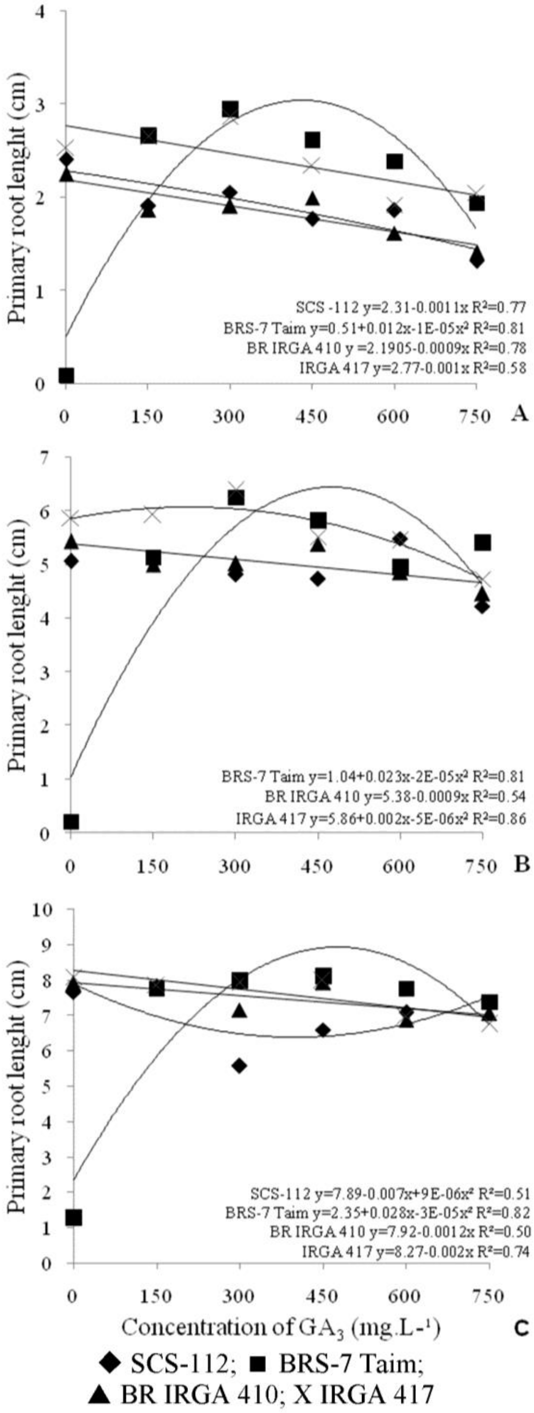

FIGURE 3 - Effect of the GA_3 concentration on seedlings primary root length of irrigated rice cultivars grown under different temperatures, at three days after sowing. 15 ºC (A); 20 ºC (B) and 25 ºC (C).

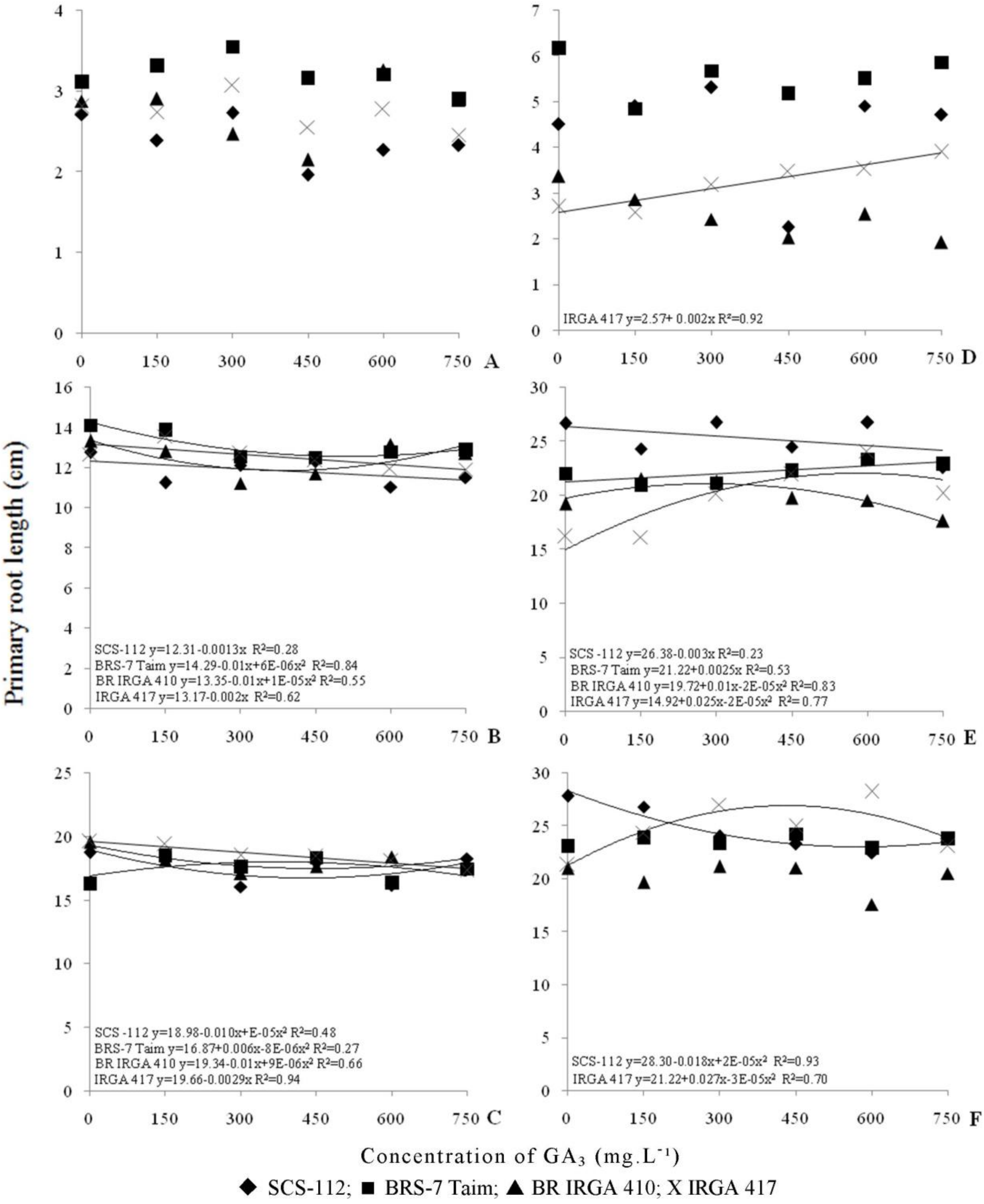

FIGURE 4 - Effect of GA_3 concentration on seedlings primary root length of irrigated rice cultivars, grown under different temperatures, at 7 and 21 days after sowing (DAS). 7 DAS: 15 ºC (A); 20 ºC (B); 25 ºC (C); and 21 DAS: 15 ºC (D); 20 ºC (E); 25 ºC (F).

The primary root length of all cultivars was not affected by gibberellin concentration at 7 DAS, when grown at a temperature of 15 ºC (Figure 4A). At 20 ºC cultivars SCS-112, BR IRGA 410 and IRGA 417 presented a slight inhibition on primary root growth with increasing GA_3 concentrations (Figure 4B). BRS-7 Taim exhibited a trend to enhanced root growth at concentrations above 600 mg L^{-1} of gibberellic acid. Similarly, at 25 ºC BRS-7 Taim responded up to concentrations of about 500 mg L^{-1}, while the rest of the cultivars experienced a mild reduction in root length with increasing GA_3 concentrations (Figure 4C).

At 21 days after sowing cultivar performance differed from that observed at three and 7 DAS (Figures 4D, 4E and 4F). In short, cultivar response varied according to temperature and concentration of gibberellic acid. At 15 ºC cultivar IRGA 417 exhibited a positive linear response in root length to increasing GA_3

concentrations, yet, the other cultivars were not affected by GA_3 (Figure 4D). Positive responses on seedling root length to GA_3 when grown at 20 ºC were recorded for genotypes BRS-7 Taim, BR IRGA 410, and, especially IRGA 417, with maximum length increases varying according to the cultivar. However, a different pattern was observed for cultivar SCS-112, with root growth linearly and negatively correlated with increases in gibberellin concentration (Figure 4E). Finally, at 25 ºC, BR IRGA 410 and BRS-7 Taim did not respond to gibberellin, while SCS-112 and IRGA 417 maintained the same response pattern observed when grown at 20 ºC. For SCS-112 decreased root growth was observed when GA_3 concentrations increased, nevertheless, for concentrations next to 700 mg L^{-1} a slight increase in root growth was observed, while IRGA 417 maintained a positive response, however, with maximum response at a lower GA_3 concentration than that recorded at the temperature of 20 ºC, between 400 and 500 mg L^{-1} (Figure 4F).

Root growth for cultivar BRS-7 Taim was barely noticeable at three days after sowing in the absence of gibberellic acid, but when subjected to the growth regulator, it showed an enhanced response up to concentrations of about 450 mg L^{-1}. Although the effect of GA_3 on shoot growth in plants is well accepted, until the moment it is known that the gibberellins have little action on the root system; nevertheless, extreme dwarf plants (lacking GA_3) also have short roots and the application of GA_3 to them promotes not only the shoot, but also root growth, not being quite elucidated whether gibberellin has a direct or indirect effect over the roots (TAIZ; ZEIGER, 2013). It should also be noticed that there is a strong interaction among plant hormones (SANTNER et al., 2009; LINKIES; LEUBNER-METZGER, 2012) and Steffens et al. (2006) showed that gibberellin acting on its own was not very effective, but acted synergistically in conjunction with ethylene, promoting an increase in the number and growth rate of adventitious roots in rice.

Regarding the inhibitory effect of root growth induced by GA_3 in cultivars SCS-112, BR IRGA 410 and IRGA 417, there is evidence that one of the locations for gibberellin synthesis is the roots (GUERRA, 2004), so that possibly the levels synthesized in this organ are enough for its normal growth, in which case the exogenous supply would raise the levels of the phytohormone to harmful concentrations. Besides, the negative effect on roots also seems to be indirect, due to a more intense shoot growth resulting in a higher seed reserve consumption, which eventually shortened their supply to the roots, thus reducing their growth rate. This was also noticed by Grohs et al. (2012), stating that it was highly detrimental to rice establishment in the field.

As mentioned regarding the effect of GA_3 on root growth, the action of a growth regulator is dependent on its concentration in the plant tissue in relation to other plant hormones, with which it interacts (GUERRA, 2004). The abscisic acid, for example, is antagonistic to gibberellin, so a higher concentration of the former precludes germination in several species (LINKIES; LEUBNER-METZGER, 2012). Synergy between auxins and gibberellins on cell expansion is well acknowledged, where the former presumably contributes to the increase on cell wall flexibility (GUERRA, 2004). Interactions with positive effect may also happen between fitohormones and other substances, as Chen et al. (2005) found that the combination of GA_3 and glycinebetaine was most effective that the treatment with only GA_3 in promoting the germination and initial growth of rice seedlings under cold stress, the same abiotic stress studied in this work. In fact, seed germination and the initial growth and development of seedlings are complex processes, where gibberellins play important roles in a diversity of steps (AN; LIN, 2011).

Regarding the synergistic effect of different growth regulators and other substances with gibberellin, a current trend in agriculture is the use of compound products, containing more than one substance, known as biostimulants, of which positive results of their in seed treatment have been already published (BERTOLIN et al., 2010).

CONCLUSIONS

Rice cultivars have different sensibility to exogenous gibberellin concentrations via seed treatment, reflecting upon seedling performance, according to the growth temperature.

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