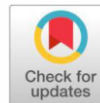


## Research Article

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# Characterization of weedy rice genotypes and study the impact of bioregulators (IAA and GA<sub>3</sub>) on seed germination of weedy rice in Jammu region



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## ABSTRACT

A Present investigation was conducted on "Characterization of weedy rice genotypes and study the impact of bioregulators (IAA and GA<sub>3</sub>) on seed germination of weedy rice genotypes in Jammu region". Three different genotypes of weedy rice were identified in farmers's fields viz. Awnless brown husk, Awns brown husk and Awnless dark brown husk. The present study was carried out on weedy rice to study their characterization on the basis of morphology, physiology, biochemical and yield attributes parameters present in the Jammu region. Two bioregulators IAA and GA<sub>3</sub> were used to examine the effect on seed germination of different genotypes of weedy rice that were treated with various concentrations of IAA (0.1 ppm, 0.5 ppm, 1.0 ppm, 2.0 ppm, 2.5 ppm) and GA<sub>3</sub> (05 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm) along with control. The results obtained from the study revealed that the plant height (cm) and the number of tillers were noticed significantly higher in Awnless brown husk (150.66 cm and 19.0). Number of grains/panicle and grains yield/plant was also increased significantly in the Awnless brown husk (225.0 and 6.41 g). Chlorophyll (SPAD value), chlorophyll 'a', chlorophyll 'b' and total chlorophyll was observed significantly higher in Awnless brown husk (67.63, 3.47 mg/g FW, 0.62 mg/g FW and 4.09 mg/g FW). Impact of bioregulators (IAA and GA<sub>3</sub>) on days required for seed germination of 1%, 50% and 100% was significantly lower when plants treated with GA<sub>3</sub>@25 ppm (2.66, 5.33 and 10.77). Seedling length (cm) was maximum in plants treated with GA<sub>3</sub> @25ppm (9.81cm ) as compared to control (7.44 cm). Therefore, it has been concluded that among all the treatments of IAA and GA<sub>3</sub>, seeds treated with GA<sub>3</sub> @ 25 ppm performed better results and germinated weedy rice seeds in the field early as compared to other treatments.

**Keywords:** Weedy rice, Rice, Bioregulators, IAA, GA<sub>3</sub>, Awnless, Chlorophyll a, Chlorophyll b, and SPAD

## 1. Introduction

Rice (*Oryza sativa* L.) is the staple food of about 3.5 billion people in the global population. Rice, being a short-saturated crop grown during the warm climate in moist and flooded soil conditions, experiences very severe competition from weeds [1]. More than 700 million tonnes of grain are produced annually from the 160 Mha of harvested rice paddies, with 90% of the area and output occurring in Asian nations. The output of rice must rise by 1% year over the next 30 years to keep up with population growth as it is currently trending upward. During the year 2020-2021, the production of rice in the world was around 515.05 million tonnes, and the area under rice cultivated is 165.25 million ha. USDA (United States Department of Agriculture) estimates that the world rice production during 2022-2023 will be 503.27 million metric tonnes [2]. In India, the production of rice increased from 53.6 million tonnes in 1980 to 120 million tonnes in 2020-21. In 2022-2023, a record 135.52 million tonnes of rice are expected to be produced. During 2021-22, the states with the largest production of rice are Uttar Pradesh (16762.2 thousand tonnes), West Bengal

(15273.4 thousand tonnes), Punjab (12885 thousand tonnes), Odisha (9136 thousand tonnes), Tamil Nadu (8067.3 thousand tonnes), Chhattisgarh (7897.7 thousand tonnes), Haryana (4618 thousand tonnes). In Jammu and Kashmir, the area under rice cultivation during 2021-22 is 280.5 thousand ha and production is 581.5 thousand tonnes. Production has increased as a result of higher yields and the cultivation of rice on more land over the past five years. China and India produce almost half of the world's rice [3].

Of late, weedy rice infestation has become a global issue in the traditional rice belts of the country. Weedy rice is one of the most notorious weeds in rice-growing regions around the world and it is a major issues. Red rice is the common name for weedy rice. It belongs to the Poaceae family, and grows alongside farmed rice. Chromosomes number is 12 (2n=24). The taxonomy of weedy rice is the same as that of cultivated rice (*Oryza sativa* L.) however it differs significantly from that species due to its seed breaking and dormancy. In comparison to cultivated rice, weedy rice is highly competitive. It has a great potential to compete against cultivated rice and significantly reduces yield. It evolved largely by natural hybridization between wild and cultivated rice, and is an emerging threat to rice cultivation as it affects crop production, harvest, quality, and income [1]. In general, weedy rice is taller and more intensively tillered. Typically, it is 40% to 57% taller than cultivated rice. Weedy rice has weaker culms and is more open or spreading. Compared to cultivated rice, which has a straw-colored hull, purple apex, and no awns, weedy rice has a black hull, purple awns, and other

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wild-like characteristics [4]. According to [5], the morphological characteristics of weedy rice are fall between the wild rice species and cultivated rice (*Oryza sativa* L.). Weedy rice has different biological traits including short growth period, black hull, strong granulation, long dormancy period, long awning, and red pericarp [6]. Red rice is a common name for weedy rice because it typically has a colored hull and a red pericarp [5]. The layers of the weedy rice caryopsis are the pericarp, seed coat, nucellus, aleurone layer, and endosperm [7]. The colored pericarp is a key characteristic that distinguishes it from typical cultivated rice [8]. It is a harmful weed that competes with rice farming for nutrients and is detrimental to rice production. It is prevalent throughout the world's rice-growing regions, especially in South and South-east Asia, South and North America, and Southern Europe. Weedy rice has become a common weed species and a significant danger to rice production in nations (such as Malaysia, Sri Lanka, Thailand, Vietnam, and the United States) where direct-seeding is the predominant technique of rice establishment. In recent years, weedy rice has been more frequently identified as a significant weed issue in Asian nations like Malaysia, Sri Lanka, Japan, China, Thailand, and India. The size of the weedy rice-affected areas differs amongst the nations. Weedy rice has a major impact on more than 2 million acres in Thailand. There are also vast paddy fields in Sri Lanka that are infested with weedy rice [9]. There are several reports of weedy rice infestation, although the percentage of contaminated areas varies. Infestations of weedy rice are found in Europe (40–70%), Italy (70%), Malaysia (74%), United States (60%) and Brazil (40%) worldwide [10]. Direct-seeded rice (DSR) has been claimed to have suffered a 74% yield reduction in Malaysia. 80 percent of the population is heavily infested (with 21 to 30 weedy panicles). In India, where DSR has been used for a long period, weedy rice is common in areas of the rice production areas in the rainfed upland rice ecosystems of eastern Uttar Pradesh, Bihar, Odissa, Manipur, West Bengal, and the hilly tracts of the northeast. India is currently experiencing a 24.28 percent in the grower's field. Weedy rice was discovered to be a significant issue in Kerala's rice fields. According to the degree of the infestation, recent heavy infestation in Kerala's crops has resulted in a 30–60% output drop. Recently, Jharkhand reported weedy rice infestations in the areas of Ranchi, Khunti, and East Singhbhum with an estimated production loss of 10–15%. In eastern and southern India, where direct seedling of rice is widespread, there is a severe infestation of weedy rice. In Haryana and Punjab, weedy rice infestation is found least because paddy is grown using the transplanting rice [11]. By using bioregulators, yield can be improved without environmental cost, which is one of the most crucial factors for scientists working around the world to ensure the availability of food for future generations. GA helps to promote seed germination, fruit growth, flowering, stem elongation, and reaction to abiotic stress. According to [12], the effect of GA treatments varies on the species, concentrations employed, and time of application. The most prevalent auxin-class phytohormone, indole-3-acetic acid (IAA), is essential for plant growth and development activities such cell division, cell expansion, cell differentiation, and fruit production. IAA homeostasis is essential for maintaining the hormonal balance at an optimum level suitable for normal plant growth and development. The physiological processes of plants can be inhibited by excessive IAA concentrations. According to several researches, IAA reduces plant development and seed germination at high concentrations [13].

## 2. Materials and Methods

Research experiments on Characterization of weedy rice genotypes and study the impact of bioregulators (IAA and GA<sub>3</sub>) on seed germination of weedy rice genotypes in the Jammu region was carried out at the Farmer's field and in the Laboratory of Plant Physiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Science & Technology of Jammu, Main Campus Chatha, during year 2022–2023. Survey has been done in the farmer's field of the Jammu region and the experimental field of SKUAST-Jammu. Three different genotypes of weedy rice were identified viz, Awnless brown husk, Awns brown husk, and Awnless dark brown husk. The present study was carried out on weedy rice to study their characterization on the basis of morphology, physiology, biochemical and yield attributes parameters present in the Jammu region. Two bioregulators IAA and GA<sub>3</sub> were used to examine the effect on seed germination of different genotypes of weedy rice, where they were treated with various concentrations of IAA (0.1 ppm, 0.5 ppm, 1.0 ppm, 2.0 ppm, 2.5 ppm) and GA<sub>3</sub> (05 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm) along with control. The experiment was Completely Randomized Design (CRD) having three replications of each treatment. Different observations were recorded as plant height (cm), number of tillers, number of grains/panicle, grains yield/plant (g), chlorophyll content by SPAD, Chlorophyll a, b, and total chlorophyll content (mg/g F.W), the impact of bioregulators (IAA and GA<sub>3</sub>) on days taken for 1, 50, 100% seed germination and seedling length (cm) of genotypes of weedy rice.

## 3. Results and discussion

### *Plant height (cm) of different genotypes of weedy rice at maturity stage*

Three genotypes of weedy rice differ significantly in terms of increasing the plant height (cm) shown in (Table 1). Plant height is an essential parameter reflecting the vertical growth of a plant. The maximum height of the plant was recorded in the Awnless brown husk (150.66 cm) followed by Awns brown husk (146.33 cm) in contrast to the Awnless dark brown husk (129.00 cm). The difference in the plant height was due to genetic character and variation of the genotype. Weedy rice is taller than cultivated rice making it more efficient for nutrients, light, and space. It has higher nitrogen use efficiency causing greater yield loss. It grows fastly and competes with cultivated rice. Similar results were reported by Shrestha *et al.* [14] revealed that blackhull weedy rice accessions have a range of plant height from 75 cm to 190 cm. Similarly, Anjali *et al.* [15] reported that the height of cultivated rice varieties "Jyothi-Ptb39" and "Uma-MO-16" was 96 cm and 91 cm, respectively, while the plant height of weedy rice morphotypes ranged between 105 to 115.67 cm

### *Number of tillers of different genotypes of weedy rice at maturity stage*

Number of tillers of weedy rice genotypes shown in (Table 1) was significantly higher in the Awnless dark brown husk (19.00) followed by the Awnless brown husk (16.33) in comparison to the Awns brown husk (14.33). The higher tillering ability of weedy rice than cultivated rice is related to a more efficient use of nitrogen and a greater capacity to direct nutrients toward shoot production. Weedy rice can produce more tillers than cultivated rice. Moreover, the high tiller production of weedy rice is correlated with its higher root surface and its greater ability to use its root architecture as a response to nutrient

stress. Rathore *et al.* [16] supported these findings and observed the same results, the total amount of tillers that the populations of Indian weedy rice produced varied significantly, but they produced more number of tillers as compared to cultivated rice that had been grown under controlled conditions.

#### **Number of grains/ panicle and grain yield/plant (g) of different genotypes of weedy rice at maturity stage**

Number of grains/panicle was significantly maximum (Table 2) in the Awnless brown husk (225.00) followed by Awns brown husk (206.00) in comparison to the Awnless dark brown husk (153.00). While, grain yield/plant (g) showed in (Table 2) was significantly higher in the Awnless brown husk (6.41 g) followed by Awns brown husk (5.49 g) as compared to the Awnless dark brown husk (4.93 g). Similar results were reported by Roy *et al.* [17] grain per panicle is high in weedy rice (117.10 grain/panicle) but very less in wild rice (39.80 grain/panicle). According to Karnet *et al.* [18] reported that the weedy rice biotypes produced 45% to 57% higher grain yield per plant than M-206 rice under competitive conditions.

#### **Chlorophyll content by SPAD, Chlorophyll 'a', 'b', and total chlorophyll(mg/g F.W) of different genotypes of weedy rice at the vegetative stage**

Chlorophyll content can be indirectly measured using a portable chlorophyll meter such as the SPAD-502. SPAD value depicted in (Table 3) was significantly higher in the Awnless brown husk (67.63) and Awns brown husk (59.16) in comparison to the Awnless dark brown husk (52.26). Rathore *et al.* [16] supported these findings. Data depicted in (Table 3) showed that the mean value of chlorophyll 'a', chlorophyll 'b' and total chlorophyll. Chlorophyll 'a' was significantly maximum in the Awnless brown husk (3.47 mg/g F.W) followed by the Awns brown husk (2.52 mg/g F.W) and Awnless dark brown husk (2.42 mg/g F.W). Similarly, the maximum value of chlorophyll 'b' was observed in Awnless brown husk (0.62 mg/g F.W) followed by Awns brown husk (0.57 mg/g F.W) and the minimum value was noticed in Awnless dark brown husk (0.50 mg/g F.W), Total chlorophyll value significantly increased in Awnless brown husk (4.09mg/g F.W) and Awns brown husk (3.09mg/g F.W) in comparison to Awnless dark brown husk (2.92mg/g F.W)

#### **Impact of bioregulators (IAA and GA<sub>3</sub>) on days taken for 1%, 50%, and 100% seed germination of weedy rice genotypes.**

Data recorded in (Table 4) reveals that days taken for 1% germination showed a significant decrease in GA @ 25ppm is 2.66 and the maximum was found in IAA @1.0 ppm is 4.55 as compared to control (4.77 days). Similarly, in (Table 5) shows days to 50 % germination was significantly decreased in GA @ 25ppm (5.33) and significantly increased in IAA @ 1.0 ppm (7.88) in comparison to control (8.00). Whereas, the result in (Table 6) revealed that for days to 100% germination showed a significant decrease in GA @ 25ppm followed by GA @ 20ppm (10.77 days), and the maximum was found in IAA @ 0.1 ppm (14.33 days) as compared to control (13.00 days). This is due to the fact that, GA<sub>3</sub> is responsible for the seed germination. GA<sub>3</sub> is a natural plant hormone that can be used to speed up the germination of seeds. The signaling pathway of GA hormone can stimulate the seed germination through the release of coat dormancy "weakening of endosperm" & expansion of embryo cells. It induces the production of hydrolyzing enzymes. These hydrolyzing enzymes in the endosperm are produced, and they stimulate the production of digestive enzymes like proteases,

amylase, and lipase which helps to mobilize stored nutrients and induce seed germination. While, auxin (IAA) is a plant hormone known to play its role in the maintenance of seed dormancy by the stimulation of abscisic acid. This hormone is responsible for the induction of seed dormancy. It promotes seed dormancy and inhibits the seed germination by enhancing ABA action. These results and findings were supported by Yan *et al.* [19] reported that for direct-seeded rice, GA<sub>3</sub> can act as a buffer to maintain good germination and a strong stand establishment. White *et al.* [20] showed that a gibberellin deficiency during seed development suppressed vivipary inABA-deficient maize kernels. Lee *et al.* [21] revealed that an excessive levels of indole-3-acetic acid (IAA) frequently cause the inhibition of plant growth and development.

#### **Impact of bioregulators (IAA and GA<sub>3</sub>) on seedling length (cm) of weedy rice genotypes.**

In the present investigation, (Table 7) showed that the impact of IAA and GA on seedling length (cm) of weedy rice was significantly increased in GA @ 25 ppm is (9.81) followed by GA@ 20 ppm and GA@ 15 ppm is (8.95) and minimum was found in IAA @ 0.1 ppm (3.47) in relation to control (7.44). This is due to GA is responsible for the growth and development of plants. Increase in height is perhaps the most widely observed effect of gibberellic acid on plants. This is mainly due to longer internodes, and not to a larger number of nodes. The increased length of an internode has been variously increasing in number of cells, length of cells, and to both. While, IAA generally inhibits the plant growth and development. IAA is the main auxin in plants, regulating growth and developmental processes such as cell division and elongation, tissue differentiation, apical dominance, and responses to light, gravity, and pathogens. These results and findings were supported by Shahzad *et al.* [22] reported that shoot length was dramatically reduced when plants were subjected to salt stress, although the application of GA<sub>3</sub> alleviated the damage caused due to salinity stress. The maximum values were reported for the GA<sub>3</sub>P + GA<sub>3</sub>FS treatment, and plants treated with GA<sub>3</sub> had longer and wider shoots than the control. Hanaa and Safaa, [23] reported that the plant maturity height (cm) revealed the presence of the plant growth regulator (IAA) at various growth stages.

**Table 1: Plant height (cm) and Number of tillers of different genotypes of weedy rice at maturity stage.**

Genotype	Plant height (cm)	Number of tillers
Awnless brown husk	150.66	16.33
Awns brown husk	146.33	14.33
Awnless dark brown husk	129.00	19.00
CD at 5%	17.02	3.46
SE(m)	4.82	0.98
C.V.	5.88	10.26

**Table 2: Number of grains/ panicle and Grain yield/ plant of different genotypes of weedy rice at maturity stage.**

Genotype	Number of grains/panicles	Grain yield (g/plant)
Awnless brown husk	225.00	6.41
Awns brown husk	206.00	5.49
Awnless dark brown husk	153.00	4.93
CD at 5%	29.98	0.93
SE(m)	8.49	0.26
C.V.	7.56	8.17

**Table 3: Chlorophyll content by SPAD, Chlorophyll 'a', Chlorophyll 'b', and total chlorophyll (mg/g FW) of different genotypes of weedy rice at vegetative stage.**

Genotype	Chlorophyll content by SPAD	Chlorophyll 'a' (mg/g F.W)	Chlorophyll 'b' (mg/g F.W)	Total Chlorophyll (mg/g F.W)
Awnless brown husk	67.63	3.47	0.62	4.09
Awns brown husk	59.16	2.52	0.57	3.09
Awnless dark brown husk	52.26	2.42	0.50	2.92
CD at 5%	5.28	0.09	0.08	0.08
SE (m)	1.49	0.04	0.03	0.03
C.V	4.34	6.12	8.51	10.20

**Table 4: Impact of bioregulators on days taken for 1 % germination of weedy rice genotypes in Jammu region chlorophyll (mg/g FW) of different genotypes of weedy rice at vegetative stage.**

Treatments	Awnless brown husk	Awns brown husk	Awnless dark brown husk	Mean
T1 (IAA 0.1 ppm)	4.33	5.33	4.66	4.77
T2 (IAA 0.5 ppm)	4.00	3.66	3.66	3.77
T3 (IAA 1.0 ppm)	4.66	4.33	4.66	4.55
T4 (IAA 2.0 ppm)	4.33	4.00	4.00	4.11
T5 (IAA 2.5 ppm)	3.33	2.66	5.33	3.77
T6 ( GA <sub>3</sub> 5 ppm)	3.66	3.33	3.33	3.44
T7 ( GA <sub>3</sub> 10 ppm)	3.66	3.33	2.66	3.22
T8 ( GA <sub>3</sub> 15 ppm)	4.00	3.66	3.66	3.77
T9 ( GA <sub>3</sub> 20 ppm)	3.00	2.66	2.66	2.77
T10(GA <sub>3</sub> 25 ppm)	3.33	2.33	2.33	2.66
T11 (control)	5.33	4.33	4.66	4.77
Mean	3.78	3.66	3.84	
CD at 5%	Genotypes = 0.64 Treatment = N/S Genotype x treatment = 1.11			

**Table 5: Impact of bioregulators on days taken for 50 % germination of weedy rice genotypes in Jammu region**

Treatments	Awnless brown husk	Awns brown husk	Awnless dark brown husk	Mean
T1 (IAA 0.1 ppm)	8.33	8.00	7.66	8.00
T2 (IAA 0.5 ppm)	7.33	7.66	7.66	7.55
T3 (IAA 1.0 ppm)	8.33	8.00	7.33	7.88
T4 (IAA 2.0 ppm)	7.33	8.00	8.00	7.77
T5 (IAA 2.5 ppm)	6.33	7.33	7.66	7.11
T6 ( GA <sub>3</sub> 5 ppm)	7.66	6.66	6.33	6.88
T7 ( GA <sub>3</sub> 10 ppm)	6.66	7.33	6.00	6.66
T8 ( GA <sub>3</sub> 15 ppm)	5.66	5.66	8.33	6.55
T9 ( GA <sub>3</sub> 20 ppm)	5.00	6.33	7.33	6.22
T10(GA <sub>3</sub> 25 ppm)	5.66	5.00	5.33	5.33
T11 (control)	7.66	8.33	8.00	8.00
Mean	6.90	7.12	7.24	
CD at 5%	Genotypes = 0.79 Treatment = N/S Genotype x treatment = 1.37			

**Table 6: Impact of bioregulators on days taken for 100 % germination of weedy rice genotypes in Jammu region**

Treatments	Awnless brown husk	Awns brown husk	Awnless dark brown husk	Mean
T1 (IAA 0.1 ppm)	14.00	14.33	14.66	14.33
T2 (IAA 0.5 ppm)	14.33	14.00	13.33	13.88
T3 (IAA 1.0 ppm)	13.66	13.33	14.33	13.77
T4 (IAA 2.0 ppm)	14.33	13.00	13.00	13.44
T5 (IAA 2.5 ppm)	13.33	13.66	14.00	13.66
T6 ( GA <sub>3</sub> 5 ppm)	13.66	12.66	13.66	13.33
T7 ( GA <sub>3</sub> 10 ppm)	8.00	13.00	13.33	11.44
T8 ( GA <sub>3</sub> 15 ppm)	12.33	11.33	9.00	10.88
T9 ( GA <sub>3</sub> 20 ppm)	9.33	9.00	14.00	10.77
T10(GA <sub>3</sub> 25 ppm)	13.66	9.33	9.33	10.77
T11 (control)	14.33	12.33	12.33	13.00
Mean	12.81	12.36	12.81	
CD at 5%	Genotypes = 0.79 Treatment = 0.41 Genotype x treatment = 1.38			

Table 7: Impact of bioregulators on Seedling length (cm) of weedy rice genotypes in Jammu region.

Treatments	Awnless brown husk				Awns brown husk				Awnless dark brown husk				Mean
	Days after germination				Days after germination				Days after germination				
	5	10	15	mean	5	10	15	mean	5	10	15	mean	
<b>T1</b>	1.06	3.03	4.76	2.95	0.50	2.83	3.23	2.18	3.26	5.90	6.70	5.28	<b>3.47</b>
<b>T2</b>	2.03	4.90	6.00	4.31	2.26	4.46	5.03	3.91	1.90	5.03	6.23	4.38	4.20
<b>T3</b>	2.90	5.63	6.86	5.13	1.96	5.00	5.93	4.29	1.16	3.40	4.00	2.85	4.09
<b>T4</b>	0.50	2.50	3.43	2.14	3.46	5.40	6.73	5.19	4.23	12.16	13.93	10.10	5.81
<b>T5</b>	2.63	5.13	6.20	4.65	2.40	5.40	6.70	4.83	1.40	3.33	5.13	3.28	4.25
<b>T6</b>	2.90	12.10	13.46	9.48	2.73	6.26	7.30	5.43	2.90	5.53	6.20	4.87	6.59
<b>T7</b>	3.56	9.76	12.80	8.70	2.60	10.26	11.96	8.27	2.50	4.93	6.86	4.76	7.24
<b>T8</b>	3.40	11.33	12.60	9.11	2.73	9.53	13.70	8.65	2.26	11.90	13.13	9.09	<b>8.95</b>
<b>T9</b>	3.93	11.10	12.70	9.24	3.16	9.23	13.16	8.51	3.86	11.26	12.23	9.11	<b>8.95</b>
<b>T10</b>	3.40	9.60	13.96	8.98	4.13	12.00	14.16	10.09	3.66	12.66	14.76	10.36	<b>9.81</b>
<b>T11</b>	2.90	4.60	5.86	4.45	3.70	11.90	13.70	9.76	2.53	8.76	13.06	8.11	7.44
<b>Mean</b>				6.28				6.46				6.56	
<b>CD at 5%</b>	Genotypes = 0.39 Treatment = 0.20 Genotype x treatment = 0.68 DAG = 0.20 Genotype X DAG = 0.68 Treatment X DAG = N/S Genotype X Treatment X DAG = 1.18												

### Conclusion

The conclusions are presented here based on the current investigation's findings. It has been concluded that among all the 11 treatments of IAA and GA<sub>3</sub>, seeds treated with GA @ 25 ppm performed better results and germinated weedy rice seeds in the field early as compared to other treatments.

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### Conflict of interest

No conflicts of interest are disclosed by the author

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