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Research Article

Growth-regulating Activity of Cinnamamide and Betaine Cinnamamide on Wheat

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Abstract: A series of Cinnamamide and betaine Cinnamamide developed from the structure of cinnamic acid were synthesized. Different concentrations of Cinnamamide and betaine Cinnamamide were employed to check their effects on seed germination and early growth in wheat. The preliminary results showed that, under the condition of this experiment, target compounds have high activity in promoting seed germination, accelerating the lengths of root and shoot and improving the quality of wheat.

Keywords: Betaine cinnamamide, cinnamamide, plant growth regulator, wheat seedlings

INTRODUCTION

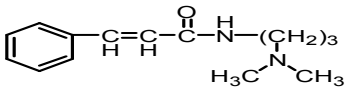
Cinnamic amide and it's derivatives are involved in a variety of activities such as anticonvulsant (Guan *et al*., 2009), anticancer (De *et al*., 2011; Zuo *et al*., 2012; Li *et al*., 2013), antidepressant (Deng *et al*., 2011; He *et al*., 1999). There were few reports about Cinnamic amide used in agricultural and it did not appear that they had been investigated for plant-growth-regulating activity.

Betaine compounds have been researched in plants (Meng *et al*., 2008; Guo *et al*., 2004). Betaine cinnamamide is a kind of quaternary ammonium salt, There were no reports about Betaine cinnamamide used as plant-growth-regulator.

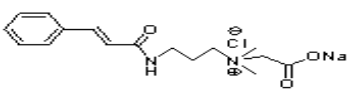
This experiment synthesis new plant growth regulators: Cinnamic amide (Fig. 1a) and Betaine cinnamamide (Fig. 1b) and studied the effects of Cinnamamide and betaine Cinnamamide on SPAD value, soluble sugar content, soluble protein content and activities of seed germination of wheat. Studying the agronomic traits and physiological changes on wheat growth, provides a theoretical basis for new substances application on improving plants growth, increase production.

MATERIALS AND METHODS

Synthesis of cinnamamide and betaine cinnamamide: Synthesis route of new compounds listed in the following Fig. 2 and 3, detail synthetic process can find from CN103694129A and CN103719082A. After the two compounds were synthesized, configured them to the concentration of 5%, respectively.



(Ia)



(Ib)

Fig. 1: Cinnamic amide (Fig. Ia) and betaine cinnamamide (Fig. Ib)

Analyses of cinnamamide: Pale yellow oily; yield: 40.0 g (98%); ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 8.244-8.246 (t, 1H, NH), 7.315-7.548 (m, 5H, Ar-H), 6.634-6.655 (2H, CH = CH), 3.875 (s, 2H, CH_2), 3.058-3.096 (q, 2H, CH_2), 2.126-2.156 (t, 2H, CH_2), 2.035 (s, 6H, CH_3), 1.474-1.530 (m, 2H, CH_2).

Analyses of betaine cinnamamide: White solid; yield: 15.0 g (86%); ^{1}H NMR (500 MHz, DMSO-d6) δ (ppm): 8.244-8.246 (t, 1H, NH), 7.315-7.548 (m, 5H, Ar-H), 6.634-6.655 (2H, CH = CH), 4.20 (s, 2H, CH_2), 3.875 (s, 2H, CH_2), 3.058-3.096 (q, 2H,CH_2), 2.126-2.156 (t, 2H, CH_2), 2.035 (s, 6H, CH_3), 1.474-1.530 (m, 2H, CH_2).

Plant material and growth conditions:

Seed germination studies: Effects of the synthesized compounds on seed germination were determined using wheat seeds. Seeds were washed with distilled water three times before use. Seeds were placed per Petri dish and soaked with distilled water and different

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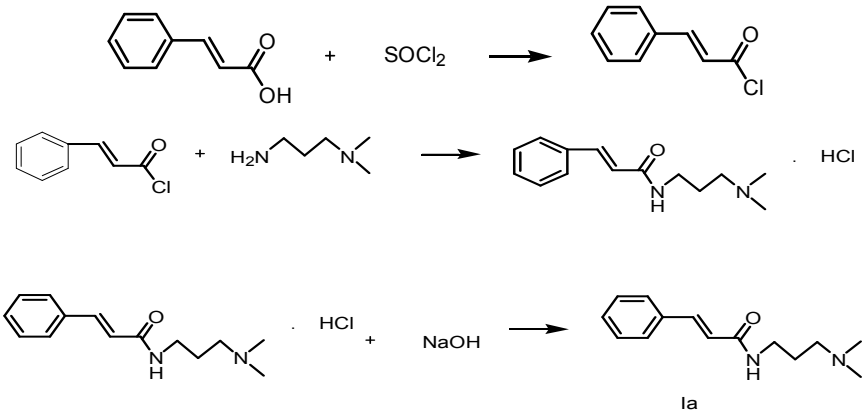


Fig. 2: Synthesis route of Ia

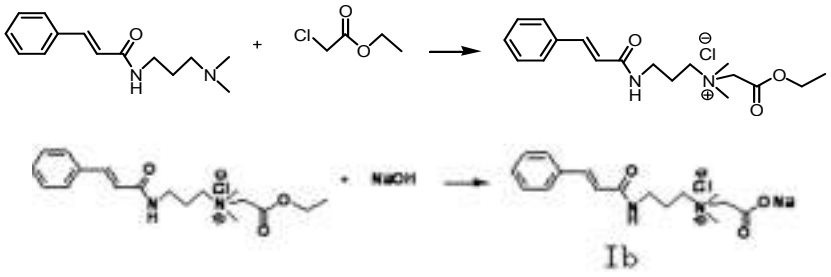


Fig. 3: Synthesis route of Ib

concentrations (10, 20, 30, 60 and 120 μg/mL, respectively) of test compounds were sprayed on double-layered filter paper. Petri dishes were placed in an incubator at 25°C, after germinating in a dark for 2 days, the number of seeds germinated was monitored. Seeds with similar-sized of radicals were chosen and transplanted to agar culture-medium. When the root and shoot lengths were long to the bottom we measured the length respectively. Control (without any chemical) were used as standards. Each experiment was performed in triplicate.

Growth activity: Pot experiment was conducted in 2013 in Qingdao Agricultural University (36.30°N, 120.36°E), Shandong Province, in northwest China. The seedling pot were PP products (150×132×110 cm), nursery substrates were: brown soil, the organic matter was 8.50 g/kg and pH of the soil was 6.70. Adopted two compounds (5%) making 5 treatments:

- CK (Control)
- Solution of 5% of cinnamon amide 500 times diluent
- Solution of 5% of cinnamon amide 1000 times diluents
- Solution of 5% of betaine cinnamamide 500 times diluents
- Solution of 5% of betaine cinnamamide 1000 times diluent, soaking seeds with the diluents

Every pot sow 20 wheat seeds. During the growth of wheat, spraying the diluents on the leaf, respectively in 30 days. Other measures comply with the local cultivation.

At harvest each plot were randomly selected 10 (with roots), mixed into a repeat sealed plastic bags, each treatment three quarters, three repeat, immediately after sealing (into the ice box) back to the laboratory for measurement. Leaves washed with deionized water, cotton drying, the leaves along the main vein cut into two parts, removing the main vein and the remaining leaves for physiological parameters were measured. Determination of chlorophyll with acetone extraction method, soluble protein content determined by coomassie blue staining, free amino acids determined by ninhydrin colorimetric method, sugar content determined by anthrone reagent method.

Statistical analysis: All graphics and data were obtained and analyzed through Excel.

RESULTS AND DISCUSSION

Effects on seed germination: Wheat seeds were tested for the germination activity of the test compounds (data shown in Table 1).

Table 1 showed that at 20 μg/mL, both cinnamon amide and betaine cinnamamide have the highest germination-rate and germination promotion rate in wheat. Betaine cinnamamide always has higher germination-rate and germination promotion rate than

Table 1: The data of wheat seeds germination activity of the test compounds

Comp.	Concentration (μg/mL)	Germination rate (%)	Germination promotion rate (%)
Cinnamon amide	10	86.0	29.13
	20	90.0	35.14
	30	85.3	28.08
	60	78.6	18.02
	120	69.0	3.60
Betaine cinnamamide	10	89.0	33.63
	20	95.3	43.09
	30	88.3	32.58
	60	82.6	24.02
	120	75.3	13.06
Control	Ck	66.6	-

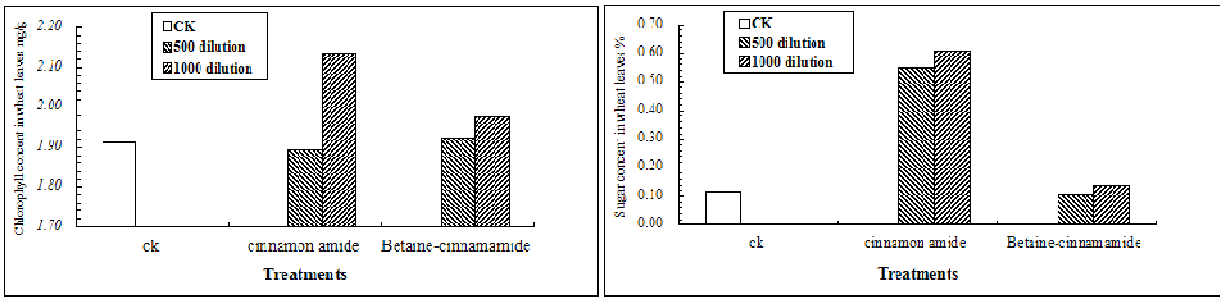
cinnamon amide at every concentration. At the same time we can see that there is a close relationship between seed germination rate and concentration.

Effects on root and shoot length: In Table 2 results listed were effects of different treatments of the new compounds on the length of shoot and root in wheat. At 30 μg/mL the two compounds have maximum length of top root (more than 60 mm), lateral root (more than 50 mm) and shoot height (more than 38 mm). For betaine cinnamamide, the maximum tap root length promotion rate (39.57%), lateral root length promotion rate (38.90%) and shoot height promotion rate (62.33%) were higher than cinnamon amide (unless the individual). And there is a phenomenon that is with the increase or decrease of concentration, the length of various determination indicator were reduced. The results showed that cinnamon amide and betaine cinnamamide have the promoting seed-germination and growth of wheat seedlings.

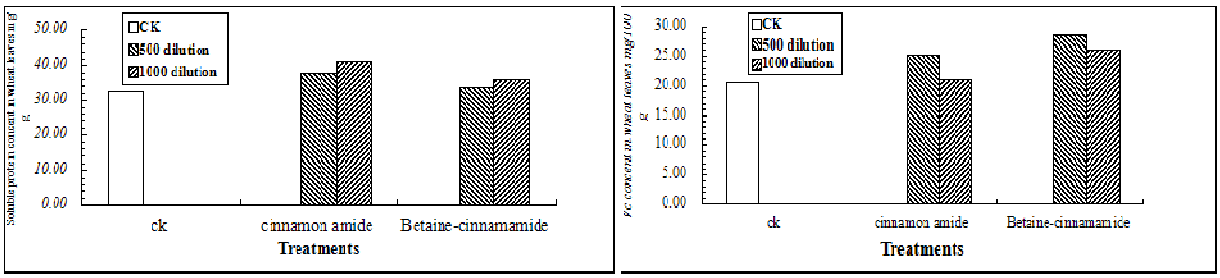
Table 2: Effects of different treatments of the new compounds on shoot length and root length in wheat

Comp.	Conc. (μg/mL)	T-r-L/mm	T-r-L P-rate/%	L-r-L/mm	L-r-L P-rate/%	S-h/mm	S-h P-rate/%
Cinnamon amide	10	48.41	2.89	39.17	2.19	25.74	5.06
	20	56.54	20.17	43.34	13.07	33.16	35.35
	30	62.65	33.16	50.21	30.99	38.74	58.12
	60	57.56	22.34	48.33	26.09	32.50	32.65
	120	52.78	12.18	45.14	17.77	29.29	19.55
Betaine-cinnamamide	10	49.40	4.99	40.17	4.80	26.76	9.22
	20	55.54	18.04	45.34	18.29	32.16	31.27
	30	65.67	39.57	53.24	38.90	39.77	62.33
	60	59.58	26.63	51.34	33.94	33.50	36.73
	120	50.79	7.95	45.17	17.85	29.24	19.35
Control	Ck	47.05	-	38.33	-	24.50	-

Tap-root Length (T-r-L); Tap-root Length Promotion rate (T-r-L P-rate); Lateral-root Length (L-r-L); Lateral-root-Length Promotion rate (L-r-L P-rate); Shoot-height (S-h); Shoot-height Promotion rate (S-h P- rate)



(a) (b)



(c) (d)

Fig. 4: Effects of different treatments on the (a) content of chlorophyll, (b) sugar content, (c) soluble protein, (d) Vc in the leaf of wheat

Fig. 5: The lettuce leaf of different treatment

Effects of different treatments on the content of chlorophyll (Fig. 4a), sugar content (Fig. 4b), soluble protein (Fig. 4c) and Vc (Fig. 4d) in the leaf of wheat: From Fig. 4, the content of chlorophyll showed a significant increase with the increase of dilution ratio for cinnamamide treatment. At 1000 times diluent, both treatment of cinnamamide and betaine cinnamamide have higher chlorophyll content than CK. For sugar content only the two treatment of cinnamamide were significantly higher than control. Figure 4c showed the content of soluble protein which were not obviously above than CK. In all process, there were a regular that the content of all indicators were 1000 dilution higher than 500 dilution, except Vc content. On proving the quality of wheat, cinnamamide have better advantage than betaine cinnamamide, but they were all good plant growth regulators for wheat.

Accompanied part of the wheat seedling roots and leaves of each treatment in Fig. 5. Through Fig. 5, the length root and shoot of every wheat seedling with different treatments can be seen clearly.

CONCLUSION

As known that wheat industry is one of the most important components of agricultural (Li, 2010; Duan *et al*., 2011; Shi *et al*., 2008), but it must meet the rapid development of the population in the limited land. Recently shortage of global energy situation deteriorated with the increasing of population. So the best way is to increase production on the basis of keeping production. In order to increase the production control the growth of plants can achieve the goal. Plant growth regulator, as a large categories of pesticides, play an important role in the agricultural of increasing production, improving the quality of plants (Ren *et al*., 2007; Xu *et al*., 2013a, b). At present the independent intellectual property rights of plant growth regulator in our country is rare, development of new plant growth regulator is imminent.

In this study, new compounds using in water-based formulations to check the activity of germination of wheat seed, rooting and Growth of the quality. The experiment results showed that the tested plant growth regulators have high activity of promoting wheat spout and rooting, at the same time, the content of SPAD, sugar, soluble protein, Vc changed obviously in the leaves of wheat. It can be seen from Fig. 5 that the wheat root of all processed developed, more fibrous root to absorb nutrients. The synthesize route of the new compounds is simple, it's dosage is safe, promoted role is good, so it was applied in field crops in Shandong and Henan province.

Due to the use of plant growth regulators is associated with many factors such as environmental temperature, dose, crop etc. So the conclusion of this test is only suitable for potting wheat plants. As for the other areas, other season, other crops the best dose of plant growth regulator application has yet to be further studied, only scientific and reasonable selection of plant growth regulator.

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