Research article

Allelopathic Activity of Peruvian Corn Varieties

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Abstract Corn (*Zea mays* L.) is one of the three most important cereals in the world. Peru is one of the centers of biodiversity of corn in the world and has 35 ecotypes. In this study we compared the allelopathic activity of Peruvian native varieties of corn using the plant box method to evaluate the activity by root exudate and sandwich method to evaluate the activity by leaf leachate for sustainable weed managements. An experiment using native varieties of 6 Peruvian corn races (Cuzco, Amarillo ancashino, Morado, Piscorunto, Chullpy, Terciopelo) was conducted at National Agrarian University La Molina, Peru. Cuzco race (known as Giant corn) was the strongest with more than 75% of lettuce radicle inhibition. Besides, these samples were evaluated by sandwich method using 10 and 50 mg of dry leaves (24 hours at 60 °C). Another experiment to compare Peruvian and Japanese varieties was conducted at National Institute for Agro-Environmental Science, Japan. A total of 85 varieties (3 Peruvian varieties and 82 Japanese varieties) and 3 types of teosinte *Euchlaena mexicana* were evaluated by plant box method. From this evaluation, Peruvian varieties of Morado (known as Purple corn) and Maiz cancha, both varieties of soft corn type, showed a strong inhibitory activity. Kuromochikibi and other Japanese varieties of waxy corn type showed also strong inhibition of lettuce radicle growth. As a conclusion, we found that Peruvian native purple corn and varieties of soft corn and waxy corn types have potent allelopathic activity and promising crop for weed control at sustainable agriculture.

Keywords *Zea mays*, allelopathy, plant box method, sandwich method

INTRODUCTION

Allelopathy is a phenomenon of interaction between the compounds emitted by plants, which can cause inhibition or promotion effects to the organisms around. These compounds are called allelochemicals and nowadays are increasing interest in use of them to suppress weeds by natural exudation. This activity was reported from some crops and the evaluation of allelopathic potential in different varieties or related species could be important in areas like plant breeding or weed management (Wu et al., 2001).

Corn (*Zea mays*) is one of the most important crops in the world. The global corn production for 2011/12 is estimated around 867.5 million tons (USDA, 2011). This crop is native from Central America and was probably introduced to Peru in an early stage. The geographical diversity generates a high variety reflected in 51 Peruvian races and other 35 ecotypes (Abu-Alrub et al., 2004; Salhuana, 2004; Sevilla, 2005). Corn is generally produced in monoculture and some cases of low production related to allelopathic effect of this crop have been reported (Sarobol and Anderson, 1992). Another research of continuous cropping of corn for 3 years showed a decrease of 13% of production compared with a field with crop rotation (Lund et al., 1993). Most research related to corn allelopathy is done with a limited number of varieties. We compare the allelopathic activity of Peruvian varieties, as well as a large number of Japanese varieties and an ancestor of corn, teosinte.

METHODOLOGY

Plant materials

First experiment: The seedlings of varieties of 6 Peruvian corn races (Cuzco, Amarillo ancashino, Morado, Piscorunto, Chullpy, Terciopelo) were cultivated in sand substrate for one month in a greenhouse of Vegetable Program of National Agrarian University La Molina (UNALM). The seeds were provided from Maize Program of UNALM. The plant box and sandwich method experiments were conducted in laboratories of the Institute of Biotechnology of UNALM.
Second experiment: A total of 85 varieties of corn (3 Peruvian and 82 Japanese varieties) and 3 types of teosinte (*Euchlaena mexicana*) were evaluated by plant box method on laboratories of National Institute of Agro-Environmental Sciences (NIAES), Japan. The seedlings were cultivated in a greenhouse with sand substrate for one month.

Plant box method

Seedlings of around one month old were used for bioassay in a Magenta GA-7 vessel (6×6×10 cm, Magenta Co. Ltd., USA) called 'plant-box', for evaluating the allelopathic activity through root exudates (Fujii, 1992). After removal of seedlings from the pot, the roots were carefully washed with distilled water and inserted into a column of a nylon mesh (0.22 mm) that was subsequently placed at a corner of the plant box. Then, autoclaved agar 0.75% (w/v) (Nacalai Tesque Co. Ltd., Japan) was placed at 40° C into the plant box. After gelatinization of the agar, a total of 33 seeds of lettuce (*Lactuca sativa* L., Great Lakes No. 366, Takii Co.) were sowed at different distances from the corner with the donor plant. The box was covered with clear wrap to prevent evaporation, and then each box was put in a black vinyl pot to cover the roots of each seedling. Finally, all the plant boxes were kept at 20 ºC with a 12/12 h photoperiod for 5 days in an incubator (BITEC-500L, Shimadzu Instruments Co. Ltd., Tokyo, Japan). After incubation, the radicle and hypocotyl length of lettuce seedlings were measured. A plant box without donor plant was used as a control. The experiment was conducted with three replications.

Sandwich method

Sandwich method was developed by Dr. Fujii as a bioassay to determinate the allelopathic activity of the leaches from donor plant leaves (Fujii, 1994). A total of 10 or 50 mg of dried leaves were placed into 3 wells of the six-well (around 10 cm^2 area per well) multi-dish plastic plate (35mm×18mm, Thermo Fisher Scientific Inc.). Agar powder (Nacalai Tesque Inc.) was used as growth medium (0.75% w/v). In each well, 5 ml of agar solution was added on 5 ml agar to make two gelatinized layers in between and 5 seeds of the test plant lettuce (*L. sativa* L., Great Lakes No. 366, Takii Co.) were seeded on the surface. The multi-dish was covered with plastic tape, labeled, wrapped in aluminum foil and incubated in dark at 25ºC for 3 days. The length of hypocotyl and radicle of lettuce seedlings were measured on the third day; these data were used to calculate the percent elongation to control.

RESULTS AND DISCUSSION

First experiment

The results of evaluation of varieties of 6 Peruvian corn races by Plant Box Method showed a strong inhibition in the lettuce radicle growth (11%) by variety of Cuzco race. The inhibition activity of other varieties ranged between 22 and 35% (Table 1).

Table 1 Evaluation of allelopathic activity of varieties of 6 Peruvian corn races by plant box method and sandwich method

Peruvian corn races	Percentage of radicle growth of lettuce(%)		
	Plant Box Method	Sandwich Method	
		10mg	50mg
Cuzco	10.9	50	21
A. Ancashino	21.9	40	17
Morado	26.6	45	21
Piscorunto	29.7	37	15
Chullpy	31.9	53	22
Terciopelo	35.0	42	13

Cuzco ('Giant corn') corresponds to the second derivation from primitive races of Peruvian corn. This race is cultivated on the Central Andes at an altitude of 2,300 to 3,300 m and was spread with the expansion of Inca Empire from Ecuador to the north of Argentina. In Peru it is mostly consumed by being boiled or as flour (Ortiz et al., 2008; Salhuana et al., 2004).

The allelopathy activity of leaves of the varieties of 6 Peruvian races was evaluated using 10 and 50 mg of dry leaves by sandwich method. By 10 mg most of them showed around 50% of inhibition of lettuce radicle growth; using 50 mg that was more than 80% in all varieties. Piscorunto showed higher activity in both concentrations; 37 and 15% growth of radicle respectively (Table 1). Piscorunto corresponds to the race derived from primitive races. This race is cultivated on the Southern Andes at an altitude of around 3,000 m (Salhuana et al., 2004).

Second experiment

The Peruvian corn varieties evaluated were Purple corn (from three commercial companies: El Shaday, Inca's Food, Peru Cheff), Cancha corn and Chullpe corn. The other group of 82 Japanese varieties was composed mostly by varieties of sweet corn. Another 3 types of teosinte, *E. mexicana*, (PI441932125, Ames8083, Ames21869) were obtained from North Central Regional Plant Introduction Station (NCRPIS).

To compare all varieties, we classified them using the description of types of corn related to the amount of starch in the grain (Tozawa, 2005).

Dent corn (*Z.mays* var. indentata): The grains have a depression on the top, with shape of tooth. Hard starch granules are accumulated in both sides and soft starch granules are accumulated from the top to the middle. Three-quarter of percentage of starch is soft.

Flint corn (*Z. mays* var. indurata): The grain has the form of hard flint, with a round shape. The soft starch is concentrated in small amount in the center covered by hard starch.

Sweet corn (*Z. mays* var. saccharata): Grain endosperm is mostly sugar. The sugar transported from leaves and stems is directly stored in the grain.

Pop corn (*Z. mays* var. everta): This group has a similar carbohydrate configuration of Flint corn, but is different in that it contains hard starch in the endosperm and a small portion of soft starch inside.

Flour corn (*Z. mays* var. amylacea): It is a round type of Flint corn. The endosperm is mostly constituted by soft starch and the grain is light.

Waxy Corn (*Z. mays* var. ceratina): This type is a mutant from China. Most of the starch consists of amylopectin with low amylose.

Table 2 Evaluation of 85 corn varieties and 3 types of teosinte by plant box method

Corn variety	Type*	Radicle growth (%)	Corn variety	Type*	Radicle growth (%)	Corn variety	Type*	Radicle growth (%)
Purple corn (El Shaday)**	Sf	3.02	Honey Bantam Peter 445	Sw	12.0	Hazetomorokoshi	P	16.9
Purple corn (Inca's Food)**	Sf	3.58	Yumemi Dream	Sw	12.2	New Dent 100 LG3457	D	17.1
Kuromochikibi	W	4.35	Peter Corn	Sw	12.5	Taiyo no megumi	Sw	17.5
Cancha corn**	Sf	4.91	Gosaku	Sw	12.6	Gold Dent KD640 [RM114]	D	18.0
Peter 001	Sw	5.06	Spectra	P	12.6	Canberra 90	Sw	18.4
Ohisama corn 7	Sw	6.00	Kyokuwase Jelly Bantam	Sw	12.9	Teosinte PI4419321250	T	19.8
Big Summer	Sw	6.74	Mirai 390	Sw	13.0	Gold Dent KD670 [MR117]	D	20.1
Ajiichiban	Sw	6.95	Mochimurasaki	W	13.1	Gold Dent KD520 [RM105]	D	20.5
Cocktail E51	Sw	7.32	Miwaku no corn Gold Rush	Sw	13.4	Canberra 86	Sw	21.6
Amaindesu	Sw	7.43	White Queen	Sw	13.8	New Dent 90 days ANJOU259	D	21.7
Oomono	Sw	7.65	Popcorn	P	14.4	Dodeka corn Yusaku	Sw	23.2
Marukajiri	Sw	8.16	Chullpe corn**	Sw	14.5	Picnic corn	Sw	23.6
Purple corn (Peru Cheff)**	Sf	8.17	Harmony Chocolate	Sw	14.9	Silage corn NS-118	D	23.7
Sunny Chocolate	Sw	8.42	SnowDent Onatsu SH9904	D	15.0	Gold Dent KD772 Super [MR130]	D	24.0
Diachi no megumi	Sw	8.79	Cocktail 600	Sw	15.0	New Dent 85 days LG3263	D	24.9
Cocktail 83L	Sw	9.23	Gold Rush	Sw	15.3	New Dent 85 days Richmond	D	25.3
Shiromochikibi	W	9.25	Woody corn	Sw	15.3	Pioneer 106 days 36B08	D	26.7
Kiimochikibi	W	9.85	Fleet	Sw	15.3	Bikkuri Sweet	Sw	27.2
Sakichan	Sw	9.94	Sweets Megumi 86	Sw	15.5	Salad corn	Sw	27.3
Ohisama corn	Sw	10.0	Honey Bantam Peter 610	Sw	15.5	Honey Bantam Wase 200	Sw	27.5
Kiihachiretsuurukibi (Longfellow)	F	10.1	Snow Dent 118 DKC61-24	D	15.5	Baby corn	P	27.7
Cocktail 84EX	Sw	10.3	Miwaku no corn	Sw	15.6	Pioneer 115 days Cecilia	D	28.4
Honey Bantam Peter corn	Sw	10.5	Teosinte Ames8083	T	15.7	Yawaraka Gold	Sw	29.3
Yume no corn	Sw	10.9	Golden Honey	Sw	15.9	Harmony Festival	Sw	29.7
Amamichan	Sw	11.2	White Popcorn	Sw	16.2	Super Suite Big	Sw	31.7
Lucy 90	Sw	11.4	Honey Bantam Peter 235	Sw	16.5	Gold Dent KD850 [MR135]	D	36.1
Sunny Fest	Sw	11.5	Cynthia Neo Dent 90 SL9945	D	16.5	Pioneer 135 days 30D44	D	40.3
Honey Bantam 20	Sw	11.7	Pioneer 88 days Deer HT	D	16.5	Pioneer 120 days 31P41	D	44.7
Yellow Popcorn	P	11.8	Super Sweet bicolor	Sw	16.8	Teosinte Ames21869	T	49.8
Honey Bantam	Sw	11.8	Amairo	Sw	16.9	Strawberry corn	P	51.1

*D Dent Corn, F Flint Corn, P Pop Corn, Sf Soft Corn, Sw Sweet Corn, W Waxy Corn, T Teosinte ** Peruvian varieties

From the total of varieties and species evaluated, the lettuce radicle growth ranged from 3 to 50%. Peruvian purple corn (El Shaday) showed the strongest inhibition effect in lettuce radicle growth (3%). Other Peruvian varieties of soft corn also showed a strong inhibition. Purple corn is considered as one of the most important Peruvian native varieties. The main characteristic of this variety is the deep purple color due to anthocyanin, present in the grains and some other parts of the ear and plant. It is cultivated from 1,200 to 4,000 m of altitude from the Coast to the Andean region. The purple corn is used to make a traditional Peruvian sweets and the pigment is used in the food industry. In recent years this compound attracts attention as a health food. (Salhuana et al., 2004; SIRA, 2005; Tenorio, 2007). The second variety with a strong inhibition was Kuromochikibi from the group of waxy type (Kuromochikibi, Shiromochikibi, Kiimochikibi). These two varieties, Purple corn and Kuromochikibi have same characteristic of dark color but it was not possible to know the relation with their inhibitory activity.

From the 82 Japanese varieties evaluated, 63% of them (52 varieties) were sweet corn type and the percentage of growth of lettuce radicle ranged from 5 to 30%. As for dent corn type, most of the varieties showed low inhibitory activity. However, to clarify whether the differences in allelopathic activity is due to genetic differences, it is necessary to explore the ancestral lineage.

Among the features in plant breeding is mentioned the reduction of the chemical components of protection (Pickersgill, 2007).

Teosinte (*E. mexicana*) is considered an ancestor of corn (Galinat, 1995). Three varieties were evaluated and two of them (Ames8083 and PI4419321250) showed a percentage of lettuce radicle growth of 16 and 20% respectively. Just Ames21869 reported a low activity (50%) compared to other types of this group.

CONCLUSION

In the evaluation of varieties of 6 Peruvian corn races by plant box, the growth of lettuce radicle fluctuated from 11 to 35% and the strongest variety was from Cuzco race. The evaluation by sandwich method of the same samples did not show significant differences.

The second evaluation of 85 varieties by plant box showed lettuce radicle growth percentage from 3 to 51%. The strongest varieties were from the group of soft and waxy corn with varieties of Purple corn (3%) and Kuromochikibi (4%) respectively.

Peruvian varieties evaluated in these two experiments showed high allelopathic activity under the plant box method. Based on these results we can affirm that allelochemicals are exuded by the roots and activity differs according to varieties. However, it is necessary to confirm the relationship between varieties and allelochemicals released from this species.

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