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Superiority of tetraploid asparagus (*Asparagus officinalis* L.) in continuous cropping

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ABSTRACT

We investigated the tolerance of two Asparagus (*Asparagus officinalis* L.) cultivars : diploid 'JG701' and tetraploid 'JGH', to autotoxicity in continuous cropping. The effects of root, foliage and rhizosphere soil extracts were tested on seed germination**.** Organic acids from rhizosphere soil were identified by HPLC. Results showed that plant fresh weight of two cultivars of asparagus decreased under replant system. However, root and shoot weights 'JGH' were higher than of 'JG701' after the second replanting. Soil extract of 'JGH' was less inhibitory to seed germination and radicle length than of 'JG701'. The tetraploid cultivar proved superior to the diploid in continuous cropping.

Key words: Allelopathy, asparagus, *Asparagus officinalis* L., autotoxicity, autotoxins, continuous cropping problem, diploid, extracts, HPLC, rhizosphere soil, seed germination, seedling growth, tetraploid.

INTRODUCTION

Autotoxicity is form of intraspecific allelopathy that occurs when planr release chemicals that inhibits or delays the germination and growth of plants of the same specie (17). Varietal allelopathy also occurs, when plants of a given variety release chemicals that inhibits or delays germination and growth of other varieties of the same crop species (14,20,22). This phenomenon is a major factor in continuous cropping problem, which causes drastic yield reduction, when same crop is planted again in the same land. For continuous cropping problem there are many reasons (i). soil-borne disease, (ii). nutritional deficiency, (iii). soil structure deterioration and (iv). autotoxicity. Autotoxins are released by the plants through root exudates, leaching, volatilization, or decomposition of plant residues and these toxins inhibit or delay the germination and growth of same plants grown in the same land.

Asparagus officinalis L. is high-value perennial vegetable crop. In recent years, its production has been rapidly increasing in China. Studies from Asia, Australia, North America, and Europe have reported that land replanted to asparagus leads to crop failure and reduced plant vigour (1,3,4,7, 16,27). Autotoxicity is one of the reasons for asparagus replanting problem (6,10,15,16,20,21,30). Unavailability of arable land in China has seriously affected the development of asparagus production industry. Autotoxins from asparagus are divided into several groups: phenolic compounds (26,27), amino acids, carbohydrates, saponins (12) and organic acids (oxalic, succinic and tartaric acids) (20).

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Diploid cultivar 'JG701'; Tetraploid cultivar'JGH'

Photograph 1. Asparagus cultivars

In the same species, there are difference in tolerance to allelochemicals among the cultivars. There are two reports, comparing the tolerance to autotoxicity between two diploid asparagus cultivars: Cultivar ‘Gijnlim' proved superior to 'UC157' in replanting conditions (19,20). The polyploid species are better adapted to wider range of environmental conditions than their lower ploidy parents (18). For example, the tetraploid and hexaploid Brassica crops have greater buffering capacity against climate change shock and adaptation to wider range of heat-stress, drought-stress or saline soil conditions (2,11).

There is little information about the tolerance of tetraploid asparagus cultivars to autotoxicity in continuous cropping system. Asparagus 'JGH' tetraploid bred in 2008, has become popular among the famous. Therefore, we want to know the superiority of this tetraploid variety over the diploid variety, so that it could provide basis for its large scale cultivation. This study aimed to overcome the replanting problem in asparagus production

MATERIALS AND METHODS

I. Study site condition

The study was conducted from April to October 2018 at Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China (Longitude : 115°56'; Latitude : 28°33'; Mean height above sea level: 40 m ; Annual Rainfall : 1700 mm; Maximum and Minimum Temperature: 38 and -3℃).

II. Plant Growth Conditions

The Jiangxi Academy of Agricultural Sciences, bred varieties 'JG701', a diploid and 'JGH', a tetraploid, were used as experimental materials in this study. Seeds of these cultivars were obtained from the asparagus research group.

III. Asparagus replanting studies

Pot culture: The experimental treatments consisted of two Factors: (i). **Cop plantings :** 3 (First planting, First Re-planting, Second Re-Replanting) and (ii). **Asparagus varieties :** 2

(Diploid JG701, Triploid JGH). The treatments were replicated thrice in randomized block design. Pre-germinated 20 days old seedlings of 'JG701' and 'JGH' were transplanted @ one seedling per pot (13 × 15 cm, containing 900 g mixture of potting mix (PINDSTRUP, Denmark) and soil (w:w 1:3) and grown for 60 days in greenhouse [25-30℃, 14:10 h (light: dark) regimes and 65-70 % humidity] in our Institute. Watering was done once in two days with 100 ml water per pot.

A modified replant culture system (20) was used to identify the growth inhibitory activity of asparagus. First planting, First replanting and Second replanting of asparagus was done on 23 April, 22 June, 21 August 2018, respectively. After 60 days growth, seedlings of both 'JG701' and 'JGH' were harvested, their roots washed, cut in pieces and air-dried. The same weight of roots in each pot) was incorporated back into the same pot soil at 2 $g \cdot kg^{-1}$ soil (20). For the second planting, 20 days old seedlings of both 'JG701' and 'JGH' cultivars were replanted in the root residue-soil mixture and grown for 60 days. Similarly, the third planting was repeated and harvested after 60 days. There was no fallow/vacant period between the harvest of preceding crop and planting of succeeding asparagus crop, i.e. the succeeding crop was sown next day after the harvest of Previous crop.

The inhibition of root or shoot growth was calculated as under:

$$\text{Inhibition}\ (\%) = (1 - T_{Fw}) / C_{Fw} \times 100$$

Where, T_{Fw} : Fresh weight of root or shoot, in first and second replanting treatments; C_{Fw} : Fresh weight of root or shoot in first planting.

Table 1. Details of Asparagus sowing and harvesting Dates

Asparagus crop	Date of sowing	Date of harvest	Growth period (days)
First planting	23.4.18	21.6.18	60
First Re-planting	22.6.18	20.8.18	59
Second Re-planting	21.8.18	20.10.18	60

There was no fallow/vacant period between the harvest of preceding crop and planting of succeeding asparagus crop i.e. succeeding crop was sown next day after the harvest of Previous crop.

IV. Soil, root and leaf Extracts

Three plants were selected from each treatment for the soil, root and leaf extracts preparation. The details of test extracts are as under:

(i). **Soil extract :** Ten g air dried soil from rhizosphere of plants was put in 100-mL conical flask in duplicate with 50 ml deionized water and shaken for 2 h on a shaker at 90 rpm. The mixture was then filtered through 2-layers of filter paper and diluted to give 0.2 and 0.3 $g \cdot ml^{-1}$ of soil extracts for assessment of their allelopathic effects on seed germination of the same cultivars.

(ii). **Roots and leaf extracts :** Air-dried roots and foliage of 3-plants from each treatment were ground to powder using Mini Grinder. Ten g powders from each treatment were put in a 100-mL conical flasks with 50 ml deionized water The flask were then sealed and placed in an ultrasonic cleaner for 1 h. The mixtures were filtered

through two-layer filter paper, followed by filtration through 0.22 µm filter using a sterile syringe. The filtrate was adjusted to 0.2 $g \cdot ml^{-1}$ and stored in refrigerator till use. When required, it was further diluted with deionized water to get 0.01, 0.03 and 0.05 $g \cdot ml^{-1}$ of root and foliage extracts. These dilutions were used to assess their allelopathic effects on seeds germination and radicle length of asparagus.

V. Petri Plate Bioassay : The experimental treatments consisted of two Factors: Extracts : 3 (Soil, root, leaf), Extracts concentrations 4: (Control, 0.01, 0.03, 0.05 g/ml). The treatments were replicated thrice in randomized block design. Asparagus seeds were sterilized by 0.03% sodium hypochlorite and soaked in sterile water for 36 h, due to seeds thick testa. For germination, 20 seeds were sown equidistant on 30 June, 2018 in Φ9.0 Petri plate on moist filter paper. The petri plates were placed in incubator [20-27 ℃, with 14 :10 h (light: dark) regimes] for 10 days.

As per treatments, 4 ml of each extract of different concentrations were added per petri plate. Sterile water served as control. The treatments were replicated thrice in randomized block design. The petri plates were kept in incubator [20-27 ℃ with 14:10 h (light:dark) regimes]. Seed germination was recorded on 7th day after the first seed germinated and the seedlings radicle length was measured at 10 d after the first seed germinated.

The results of seed germination and root growth were expressed in *RI* Value as per Williamson (23) :

If T ≥C, then *RI*=1-C/T

If T <C, then *RI*= C/T-1

Where, C : Control ; T : treatment ; *RI* : Allelopathic index. *RI* > 0 : stimulation, *RI* < 0 : inhibition.

VI. Extraction and Isolation of organic acids

One hundred mL of deionized water in 250 ml-conical flask was mixed with 30 g rhizosphere soil from 'JG701' and 'JGH' pots and placed on shaker for 2 h. Soil extract was then filtered through two-layer filters. The filtrate was first passed through cation-exchange resin column (Φ2.4 cm×80 cm) containing 300 g of cation-exchange resin). The eluent from this column was passed through anion exchange resin (300 g) column for acid separation, at flow rate of 0.5-0.6 $mL \cdot min^{-1}$. The column was then eluted with $1mol \cdot L^{-1}$ HCL, at a flow rate of 0.4-0.5 $mL \cdot min^{-1}$. The eluate, 300 ml, from this column was evaporated to dryness using rotary evaporator (temperature 30 ℃, speed 80-90 $r \cdot min^{-1}$). The dried residue was dissolved in 10 ml deionized water and filtered through 0.22 µm filter in a sterile syringe and subjected to HPLC analysis.

HPLC analysis was done using Waters 600 chromatograph fitted with a C_{18} column (150 mm-4.6 mm I.D., 5 µm); Mobile phase was A: 0.022 $mol \cdot L^{-1}$-KH_2PO_4 (PH 2.2) and Mobile phase B: 3 % CH_3OH. Flow rate: 0.7 $mL \cdot min^{-1}$ and gradient elution was done with the same concentration**.** The column temperature was 30 ℃. Injection volume was 20 µl. Eluants were monitored using a UV detector at 210 nm.

For standard curve: 10 $mg \cdot mL^{-1}$ of tartaric acid, malic acid, succinic acid and ferulic acid**,** purchased from Shenggong chemicals company, were filtered through 0.22

µm filter in a sterile syringe and subjected to HPLC analysis and the retention times of each compound were determined.

Statically Analysis of Data: The data were analyzed using a statistical package, DPS version 7.05. Differences between treatment means were tested using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Growth inhibition

The auto-toxicity was a possible reason in *Asparagus* replant problem, as the root residues in all plantings of asparagus inhibited the root and shoot growth of its own seedlings (27). Hence, in later studies, it was confirmed that asparagus is an autotoxic specie (29). Growth inhibition of Asparagus after the first and second replanting is shown in Figure 1. The inhibition of root and shoot growth was significantly higher in 'JG701' than in 'JGH'. The inhibition was more in the second replanting than in the first. In 'JG701', root growth was more inhibited (30 %) than shoot growth (23 %). A similar trend was observed in TGH, but the magnitude of inhibition in root and shoot growth was less than in JG70. The potential auto-toxicity to root and shoot growth was stronger with higher ploidy cultivar in replanting system (Figure 1).

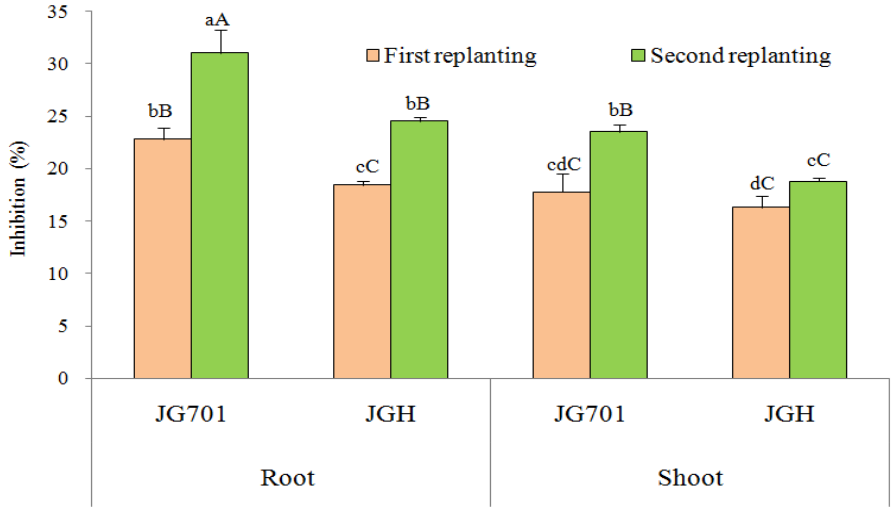

Figure 1. Root and shoot inhibition of two different ploidy asparagus cultivars in replanting system
Different lowercase letter means significant difference at 5% level, different uppercase letter means significant difference at 1% level.

Autotoxic effects of asparagus plant and soil

The parts of asparagus plants have allelopathic or autotoxic effects or its own seedlings and also on other species (5,8,19,20). Asparagus tissue extracts are toxic to the growth of asparagus and other vegetable seedlings (11). In *Malus hupehensis* continuous

cropping, the tartaric acid in the soil decreased the biomass of *M. hupehensis* seedlings (13). Soil extract from the first and the second replanting inhibited the seed germinations of both 'JG701' and 'JGH' at 0.2 g·ml^{-1} to 0.3 g·ml^{-1} concentration (Figure 2).

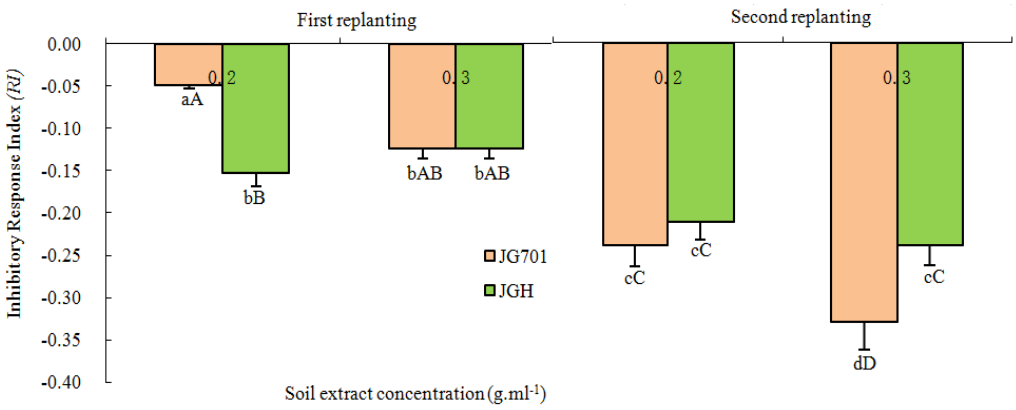

Figure 2. Response index of soil extract effects on seed germination in the replanting system
Different lowercase letter means significant difference at 5% level, different uppercase letter means significant difference at 1% level.

The inhibition in seeds germination of both 'JG701' and 'JGH' was higher in the second replanting than the first replanting. The inhibitory effect increased from 0.2 g·ml^{-1} to 0.3 g·ml^{-1} concentration in the second replanting and were concentration dependent. In cultivars, germination inhibition was more in JG701 at soil extract concentrations of 0.2 g·ml^{-1} to 0.3 g·ml^{-1}. The soil extracts from first and second replanting showed inhibitory effects on radicle growth of both cultivars (Figure 3). Extract from the second replanting soil was more inhibitory to radicle growth in both cultivars than from the first replanting. The soil extract was significantly more inhibitory to radicle growth of 'JG701' at both concentrations (0.2 g·ml^{-1} and 0.3 g·ml$^{-1)}$ than 'JGH'. Soil extract concentrations from two plantings slightly suppressed the radicle growth. Yang (24,25) examined the effects of extracts from the stems, crowns and roots of dead asparagus plants on its own seedlings of asparagus varieties ' Mary Washington', ' Viking ' and ' Welcome' (5,9,15). The aqueous extracts of dried asparagus roots contains allelochemicals viz., Ferulic, isofemlic, malic, citric and fumaric acid (5). The fraction of methanol extract of asparagus fresh root tissue was very inhibitory to asparagus seedlings contains the caffeic acid, which delayed the seedling emergence of asparagus (15). 'JGH' root extract was increasingly inhibitory to its own seed germination at 0.01 to 0.05 g·ml^{-1} concentrations (Figure 4). On the other hand, 'JG701' root extract stimulated its own seed germination at 0.01 g·ml^{-1}, but this positive effect gradually decreased. At all concentrations 'JG701' root extract was more inhibitory to its own seed germination than 'JGH'. The Asparagus seed germination at 0.01 to 0.03 g·ml^{-1} concentrations was similar in foliage extract of 'JG701' and 'JGH'. Root extract at 0.03- 0.05 g·ml^{-1} concentrations were more inhibitory to seed germination of 'JGH' than that of 'JG701.

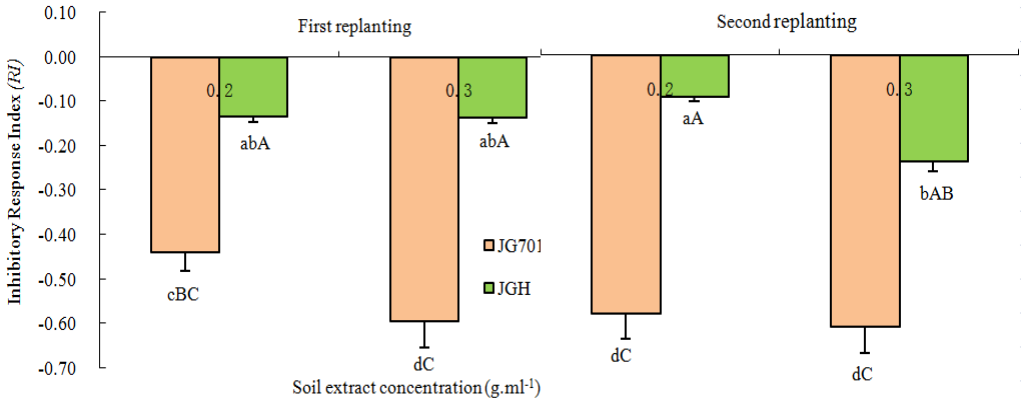

Figure 3. Response index of soil extract effects on radicle length in the replanting system
Different lowercase letter means significant difference at 5% level, different uppercase letter means significant difference at 1% level.

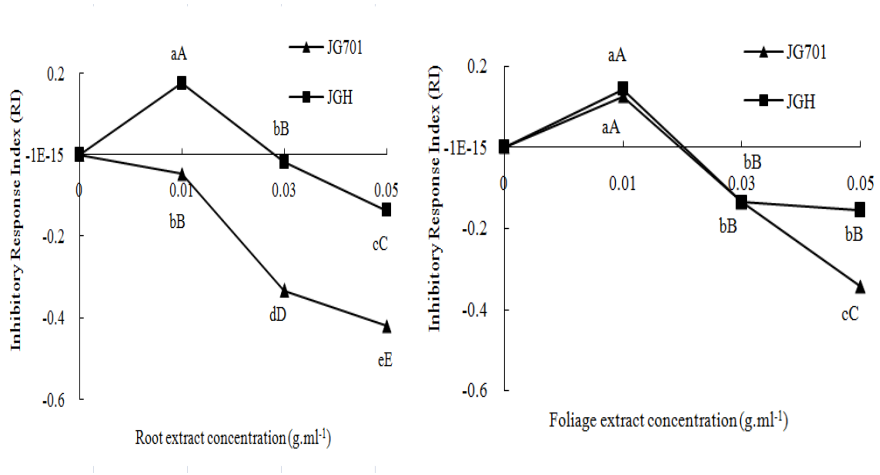

Figure 4. Effects of root and foliage extracts of asparagus on its own seeds germination
Different lowercase letter means significant difference at 5% level, different uppercase letter means significant difference at 1% level.

Chemicals in asparagus replanted soil

In three planting models, 4-organic acids (tartaric acid, malic acid, succinic acid, ferulic acid) were detected in the asparagus rhizosphere soil of both cultivars 'JG701' and 'JGH' (Table 2). In first planting, tartaric acid was absent in the rhizosphere of 'JG701', but its concentration increased in the second replanting soil. Malic acid concentration was increased more from the first planting than the first replanting and was significantly higher

in 'JG701' than 'JGH'. Succinic acid concentration was significantly higher in 'JGH' than 'JG701'. Ferulic acid increased from first planting to second replanting soil. Ferulic acid content was significantly higher in 'JGH' than 'JG701'. In summary, the organic acids concentration increased with replantings and their content was higher in 'JG701' rhizosphere than in 'JGH'. In asparagus rhizospehere soil, growth inhibitory allelopathic substances are present (16). Young (26,28) analyzed the soil extracts from asparagus grown soils, which were analyzed using XAD-resin. The phenolics fraction isolated from the asparagus soil inhibited the growth of asparagus seedlings. The trans-cinnamic acid accumulated up to 174 μM in 10-years asparagus grown soils (8). In the present study, there were higher inhibitory effects in 'JG701' than in 'JGH' (Figs 2, 3 and 4). Four organic acids (tartaric, malic, succinic and ferulic) were detected in asparagus 'JG701' and 'JGH' soil (Table 2). It is well known that higher ploid cultivars generally show stronger tolerance to environmental stress. Our present studies showed that tetraploid asparagus cultivar JGH, was less inhibitory to its own seedlings growth. Besides, crude extracts of soil and autotoxins inhibition of seed and radical were lower in tetraploid cultivar than diploid. Thus the higher ploid cultivar of asparagus showed stronger tolerance to autotoxicity.

Table 2. Organic acids detected in asparagus in replanting system soil

Cultivation model	Cultivars	Tartaric acid ($\mu g \cdot g^{-1}$)	Malic acid ($\mu g \cdot g^{-1}$)	Succinic acid ($\mu g \cdot g^{-1}$)	Ferulic acid ($\mu g \cdot g^{-1}$)
First planting	JG701	0.00dD	19.80eE	15.02dDE	2.08cC
	JGH	5.27dD	10.67eE	30.75cC	0.82eE
First replanting	JG701	218.42bB	814.69bB	12.46dE	1.41dD
	JGH	20.39dD	546.06dD	164.23bB	0.60fF
Second replanting	JG701	441.03aA	921.32aA	29.54cCD	7.23aA
	JGH	101.97cC	660.09cC	263.06aA	2.58bB

Different lowercase letter means significant difference at 5% level, different uppercase letter means significant difference at 1% level.

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