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Responses of Soybean (*Glycine max* L.) To Soil Solarization and Rhizobial Field Inoculation at Dang Ngaoundere, Cameroon

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Abstract: Field experiments were conducted to investigate the performance of two soybean (*Glycine max* L.) cultivars under soil solarization and rhizobial inoculation. Trials were carried out during the first cropping season of 2004 and 2005 at Dang Ngaoundere in the Guinea Savannah zone of Cameroon. In addition to one month soil solarization schedule performed during the first year, two and three months schedules were surveyed during the second year to identify the most efficient duration of solarization for plant growth improvement. The experimental design was multifactorial comprising three randomized factors: inoculation, cultivar and soil solarization. Results indicated that one month soil solarization was not enough to improve growth parameters of C_1 and C_5 soybean cultivars. Two months soil solarization increased plant biomass by 47 and 85%, flowering by 70 and 62% and seed yield by 75 and 85%, respectively for C_1 and C_5 cultivars compared to the control. Similarly, nodulation and seed yield were improved by rhizobial inoculation. The combined effect of two months soil solarization and rhizobial inoculation on growth parameters differed from one cultivar to another. Although three months soil solarization was the least effective treatment, it enhanced plant biomass by 14 and 32% and seed yield by 61 and 30%, respectively for C_1 and C_5 cultivars.

Key words: Biomass, inoculation, nodulation, solarization, soybean, yield

INTRODUCTION

In the Northern region of Cameroon, cereals are considered as the basic food to human and other animals. Although they provide important calories, they remain deficient in protein (Tien *et al*., 2002). Legumes constitute an excellent supplement of protein for a diet rich in cereals, because they can provide between 18 to 38% of proteins (Watier, 1982). For soybean in particular, a protein content of 45% has been reported (Roux, 2002). Despite these attributes, the constraints of soybean are among other a low yield due to low soil fertility, insect pests and diseases. Synthetic chemicals are commonly utilized to control these constraints. However, extensive uses of pesticides pose a risk for pollution of the environment and food (Fresco, 2003). The development of plant pathogen resistance to chemical compounds is another risk factor. There has been increasing demand for reduction of chemical methods for crop production, both from consumers and farmers. A few studies have indicated the possibility of rhizobial inoculants to improve soybean yield and other legumes (Gomez *et al*., 1997; Okereke *et al*., 2001). Nevertheless, this inoculants-base technology can not overcome pathologic constraints. Soil solarization is a non chemical method that heats and disinfects the soil and reduces the activity of both harmful and beneficial organisms (Braga *et al*., 2001). We hypothesize that soil solarization could inactivate soil pathogenic organisms and thus promote soybean production through inoculation techniques. The main objective of this study was to enhance soybean production in Ngaoundere through improved soil fertility by exploiting the rhizobial inoculation and soil solarization techniques.

MATERIALS AND METHODS

Location and characteristics of study area: The field trials were located at Dang-Ngaoundere in the Guinea Savannah zone of Cameroon (7°24.61'N 13°34.24'E, at 1155.8 m elevation). The soil was brown reddish developed on a basaltic rock, with pH 5.45, 0.16 g N 100 g soil, 0.04 g P 100g soil, 2.9 g total C 100 g soil.

Soybean seeds cultivars: Two soybean cultivars, C_1 and C_5 were used in this study. The C_1 cultivar is brownish in colour, produces violet flowers, greenish pods at maturity and has a growth cycle extending from 145 to 150 days.

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The C_5 cultivar matures between 155 and 160 Days After Planting (DAP) and produces white flowers and white pods at harvest.

Soil solarization: Soil solarization was performed in the field by covering each 2×4 m plot with a 2.5×4.5 m clear plastic sheeting (polyethylene) for one month in the first year and one, two or three months in the second year. The edges of the plastic sheeting were covered with soil to avoid windisturbance. To increase the efficiency of the process, soil was maintained smooth on the surface and wetted prior to solarization.

Rhizobial inoculants and inoculation technique: The inoculums made of *Bradyrhizobium* sp. was purchased from the Microbiology laboratory of the Biotechnology Centre, University of Yaounde I (Cameroon). Before rhizobial inoculation, soybean seeds were sterilized with 70% ethanol for 15 min, followed by three washes with tap water to remove traces of ethanol. Seeds (600 g) of each soybean cultivar were coated with a mixture of 100 g of powder milk (Nido), 200 g inoculum, 200 mL tap water and sown immediately at a rate of 2 seeds per stand.

Experimentation: Experimental plots were manually cleared with cutlasses and ploughed with hoes. Each plot consisted of 6 rows of 15 pockets 30 cm apart, thus 3 rows per cultivar on one side of the plot. Trials were carried out during the first cropping season of 2004 and 2005. In 2004 only one month (07/03 to 07/04/04) soil solarization was performed. In addition to one month (04/03 to 04/04/05) solarization schedule, two (04/03 to 04/05/05) and three (04/03 to 04/06/05) months were surveyed during the second year to identify the most efficient solarization time for improved growth. The experimental design was multifactorial comprising three randomized factors: inoculation, cultivar and solarization. The factor solarization consisted of covering a plot with a white plastic film for one (S_1), two (S_2), or three (S_3) months. The factor inoculation consisted of coating seeds with rhizobial inoculum and sow either on non solarized and non inoculated plots (T), non solarized plots (R) or solarized plots (RS_1, RS_2, RS_3). The factor cultivar consisted of sowing the C_1 cultivar on one side and C_5 on the other side of the same plot. Each treatment was replicated three times. At 45 and 60 Days After Planting (DAP), growth parameters were evaluated (e.g., plant biomass, inflorescences per plant, number and dry weight of nodules per plant). At harvest, seed yield was evaluated and expressed in t ha^{-1}.

Growth parameters measurements: Ten labelled plants per replicated plot were randomly selected to evaluate the growth parameters. At 50% flowering, each plant was carefully removed from the soil with a cutlass and the number of root nodules recorded. To evaluate the weight of nodules, all the root nodules collected from each plant were labelled, dried for 12 h at 60°C and weighed separately as described by Ngakou *et al*. (2003). After recording the number of inflorescences per plant, all the plants per replicate plot were also dried under the same conditions and weighed separately.

Yield evaluation at harvest: At maturity ten other plants were labelled per replicate plot and their pods were collected. Seeds from all the pods of the same replicate plot were harvested separately for the estimation of seed weight in t ha^{-1} per treatment.

Statistical analysis: Data were statistically analyzed by ANOVA using a Statgraphic plus computer program. Means were compared between treatments using the Duncan's Multiple range test at 5% level of significance.

RESULTS

Biomass of two soybean cultivars: Field experiments conducted during the 2004 cropping season indicated an inhibitory effect of treatments on plant biomass of the two cultivars (Fig. 1A). Inoculation had no effect on the biomass of cultivar C_1. The inhibitory effect was more pronounced when inoculation was combined to soil solarisation, with a 45% reduction of biomass for C_1 and 36% for C_5. We related this reduction to the insufficient duration of soil solarization (one month) and therefore, we took into consideration during the 2005 cropping season several solarization schedules (one, two and three months) to evidence the most efficient solarization time. Under these conditions, the effects of different treatments were significant at 5% level (Fig. 1B). The higher biomass was observed for two months soil solarization with 46.7% for C_1 and 84.7% for C_5. At three months soil solarisation, an inhibitory effect on plant biomass was instead observed for C_5 cultivar. This effect was accentuated when soil solarization was combined to rhizobial inoculation (a 25% reduction). Cultivar C_1 was found to respond better to treatments than C_5 during the 2005 cropping season compared to 2004 season.

Flowering of two soybean cultivars: Like for plant biomass, one month soil solarization experimented during the 2004 cropping season was not enough to significantly increase the number of inflorescences per soybean plant. Unlike, treatments significantly reduced the number of inflorescences at 5% level (Table 1). These reductions were 14% at 50% flowering and 13% at 100% flowering

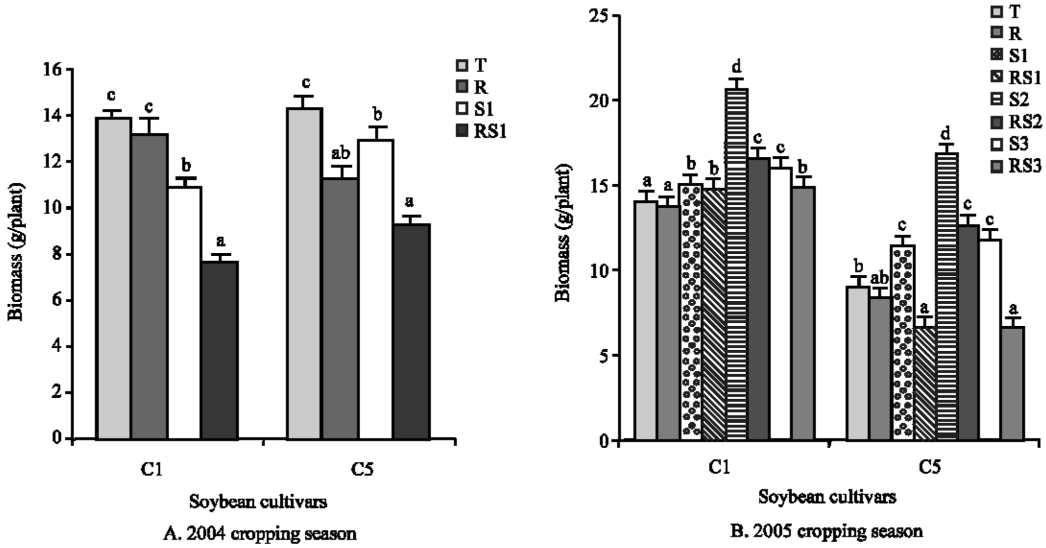

Fig. 1: Effects of different treatments on two Soybean cultivars Biomass (g plant^{-1}) T: Uninoculated and unsolarized plot; R: Rhizobial inoculated plot; S_1: One month soil solarized plot; RS_1: Rhizobial inoculated and one month soil solarized plot; S_2: Two months soil solarized plot ; RS_2: Rhizobial inoculated and two months soil solarized plot; S_3: Three months soil solarized plot; RS_3: Rhizobial inoculated and three months solarized plot; n/a: not available; Bars with different letter within a cultivar are significantly different at $p<0.05$. Error bars are standard deviation of three replicate measures

Table 1: Effects of different treatments on the number of inflorescences of the two soybean cultivars (n = 10)

Cropping years	2004				2005			
Sampling time	50% flowering		100% flowering		50% flowering		100% flowering	
Cultivars	C_1	C_5	C_1	C_5	C_1	C_5	C_1	C_5
T	17.07 ± 1.10^{ab}	20.66 ± 2.36^{b}	28.87 ± 1.56^{a}	34.16 ± 4.86^{b}	56.33 ± 1.80^{ab}	48.10 ± 3.80^{a}	70.6 ± 6.20^{a}	76.2 ± 6.70^{a}
R	17.80 ± 1.93^{b}	15.16 ± 1.92^{a}	25.87 ± 1.64^{b}	27.13 ± 1.82^{a}	31.67 ± 3.40^{a}	65.73 ± 6.40^{bc}	94.40 ± 13.00^{abc}	102.87 ± 14.00^{abc}
S_1	14.60 ± 1.64^{ab}	19.03 ± 2.32^{b}	25.20 ± 1.52^{a}	30.86 ± 3.09^{ab}	49.13 ± 3.97^{bc}	66.80 ± 5.80^{bc}	106.33 ± 8.40^{cd}	109.00 ± 9.00^{bc}
RS_1	13.60 ± 0.73^{b}	14.60 ± 1.86^{a}	25.60 ± 1.87^{a}	27.66 ± 2.77^{a}	93.60 ± 8.80^{e}	59.13 ± 8.75^{abc}	86.67 ± 4.30^{ab}	93.00 ± 4.50^{ab}
S_2	n/a	n/a	n/a	n/a	70.73 ± 6.80^{d}	70.27 ± 4.60^{c}	120.07 ± 8.90^{de}	124.00 ± 9.20^{c}
RS_2	n/a	n/a	n/a	n/a	55.53 ± 4.00^{c}	67.73 ± 4.60^{bc}	105.00 ± 12.00^{cd}	108.00 ± 13.00^{bc}
S_3	n/a	n/a	n/a	n/a	60.93 ± 4.10^{cd}	66.73 ± 6.00^{bc}	83.87 ± 11.00^{a}	85.27 ± 11.00^{ab}
RS_3	n/a	n/a	n/a	n/a	32.07 ± 4.00^{a}	52.60 ± 5.00^{ab}	88.27 ± 11.00^{ab}	87.13 ± 4.00^{ab}

T: Uninoculated and unsolarized plot; R: Rhizobial inoculated plot; S_1: One month soil solarized plot; RS_1: Rhizobial inoculated and one month soil solarized plot; S_2: Two months soil solarized plot; RS_2: Rhizobial inoculated and two months soil solarized plot; S_3: Three months soil solarized plot; RS_3: Rhizobial inoculated and three months solarized plot; n/a: not available; Value with different letter(s) in a column are significantly different at $p<0.05$

for C_1 cultivar, whereas C_5 cultivar presents 30 and 19% reduction at 50 and 100% flowering, respectively. Comparatively to the control, other treatments also reduced the plant biomass.

During the 2005 cropping season, the one month soil solarization had no effect on C_1 cultivar at 50% flowering. In contrast, the combination of rhizobial inoculation and two month soil solarization (RS_1) significantly increased the number of inflorescence of this cultivar by 66%. Generally, all the treatments increased the number of inflorescences at 50% flowering for C_5 cultivar with a maximum registered for two months soil solarisation (46%). Whereas rhizobial inoculation inhibited the number of inflorescences (-47%) for C_1 cultivar, that of C_5 cultivar was rather enhanced (37%). At 100% flowering, all the treatments significantly increased the number of inflorescences per plant. C_1 cultivar was more sensitive to treatments (with 50% increase for S_1 and 70% for S_2) than C_5 cultivar (with 43% increase for S_1 and 62% for S_2). However, three months soil solarization inhibited flowering of the two soybean cultivars. At the same flowering date, rhizobial inoculation stimulated the flowering of the two cultivars by 33% for C_1 and 35% for C_5. C_1 was a fast flowering cultivar (at 64 DAP) while C_5 cultivar flowered late (at 75 DAP).

Table 2: Effects of different treatments on nodulation of two soybean cultivars (n = 10)

Cropping years	2004				2005			
Samples	Number of nodules		Weight (g) of nodules		Number of nodules		Weight (g) of nodules	
Cultivars	C_1	C_5	C_1	C_5	C_1	C_5	C_1	C_5
T	22.27 ± 0.10^{a}	30.33 ± 1.00^{bc}	0.12 ± 1.10^{a}	0.13 ± 1.00^{a}	67.87 ± 8.453^{a}	94.24 ± 10.307^{bc}	0.30 ± 0.42^{abc}	0.30 ± 0.43^{bc}
R	60.80 ± 0.02^{b}	41.53 ± 0.00^{c}	0.34 ± 0.20^{b}	0.23 ± 0.20^{b}	79.80 ± 8.02^{ab}	92.87 ± 15.91^{b}	0.41 ± 0.46^{bcd}	0.28 ± 0.41^{abc}
S_1	20.13 ± 0.30^{a}	21.60 ± 0.10^{ab}	0.12 ± 0.13^{a}	0.11 ± 1.10^{a}	91.47 ± 7.62^{ab}	110.00 ± 13.91^{bc}	0.28 ± 0.38^{ab}	0.39 ± 0.01^{cd}
RS_1	18.20 ± 1.00^{a}	17.40 ± 0.11^{a}	0.12 ± 1.11^{a}	0.17 ± 1.10^{a}	180.47 ± 5.60^{c}	128.68 ± 10.00^{c}	0.46 ± 0.043^{d}	0.47 ± 0.06^{d}
S_2	n/a	n/a	n/a	n/a	132.80 ± 14.78^{abc}	119.73 ± 7.13^{bc}	0.46 ± 0.56^{d}	0.26 ± 0.16^{ab}
RS_2	n/a	n/a	n/a	n/a	123.27 ± 11.83^{abc}	178.93 ± 16.60^{d}	0.42 ± 0.55^{cd}	0.48 ± 0.56^{d}
S_3	n/a	n/a	n/a	n/a	135.33 ± 15.57^{bc}	$124.73\pm14.93b^{c}$	0.46 ± 0.40^{d}	0.34 ± 0.042^{bc}
RS_3	n/a	n/a	n/a	n/a	69.00 ± 15.31^{ab}	49.47 ± 1.91^{a}	0.24 ± 0.53^{a}	0.17 ± 0.07^{a}

T: uninoculated and unsolarized plot; R: Rhizobial inoculated plot; S_1: One month soil solarized plot; RS_1: Rhizobial inoculated and one month soil solarized plot; S_2: Two months soil solarized plot; RS_2: Rhizobial inoculated and two months soil solarized plot; S_3: Three months soil solarized plot; RS_3: Rhizobial inoculated and three months solarized plot; n/a: not available; Value with different letter in a column are significantly different at $p<0.05$

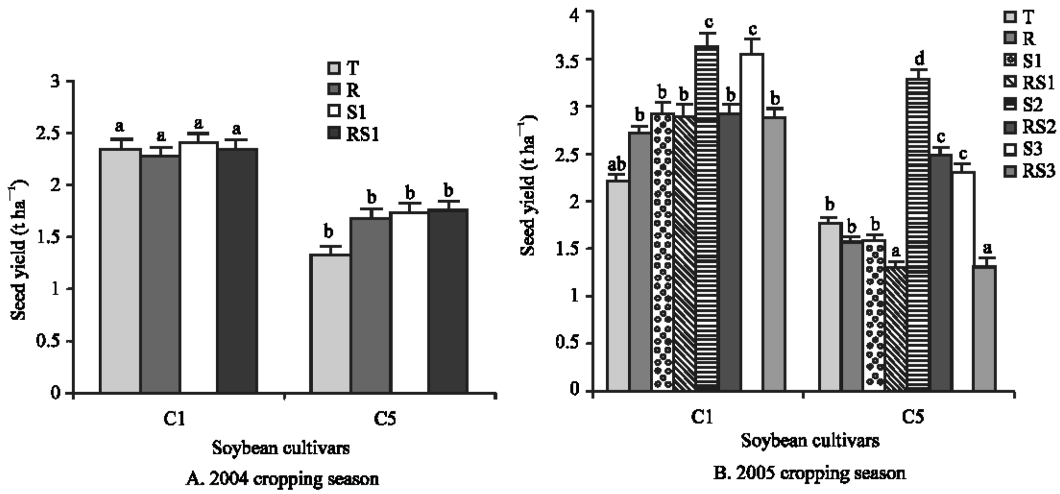

Fig. 2: Effects of different treatments on seed yield (t ha^{-1}) of two Soybean cultivars T: Uninoculated and unsolarized plot; R: Rhizobial inoculated plot; S_2: One month soil solarized plot; RS_1: Rhizobial inoculated and one month soil solarized plot; S_2: Two months soil solarized plot; RS_2: Rhizobial inoculated and two months soil solarized plot; S_3: Three months soil solarized plot; RS_3: Rhizobial inoculated and three months solarized plot; n/a: not available; Bars with different letter within a cultivar are significantly different at $p<0.05$. Error bars are standard deviation of three replicate measures

Two soybean cultivars nodulation: Experiments carried out in 2004 revealed that the number of root nodules were higher in R treatment irrespective to the cultivars (Table 2). Rhizobial inoculation increased the number of root nodules by 173% for C_1 and by 43% for C_5 cultivars compared to the control. Similarly, rhizobial inoculation enhanced the nodule dry weight by 183% for C_1 and 77% for C_5.

During the 2005 cropping season, treatment S_1 did not significantly enhance the nodule number, particularly for cultivar C_1 ($p= 0.05$). In contrast the other treatments increased the nodule number compared to the control. RS_1 treatment increased nodules number of C_1 cultivar by 166%, whereas RS_2 treatment enhanced that of C_5 cultivar by 90% (Table 2). Three months soil solarization inhibited the native strains of *Rhizobium* in the soil, so that the combination of inocution and three months solarization behave like R treatment regardless the number of root nodules (Table 2). There was a significant positive correlation between the nodulation and the number of nodules of C_5 cultivar ($r = 0.20$; $p<0.001$). Soil solarization above two months efficiently improved the number of nodules per plant relative to experiment conducted in 2004 where inhibition was observed for this parameter.

Treatments S_1, S_2, RS_1 and RS_2 contributed to 54% increased dry weight of nodules compared to the control (T) for C_1 cultivar, whereas for C_5 cultivar, treatment RS_2 was the best with 62% increased of nodule dry weight.

Two soybean cultivars yields: During 2004 cropping season, treatments significantly increased the seed yield of C_5 cultivar by 26, 30 and 32%, respectively for treatments S_1, R and RS_1 (Fig. 2A). Though different treatments did not affect C_1 cultivar, it was a high yielded cultivar than C_5. There was a significant positive correlation ($r = 0.31$, $p = 0.00$ for C_1; $r = 0.35$, $p = 0.00$ for C_5) between the seed and pod yields.

During the 2005 cropping season, C_1 remain again the high yielded cultivar (Fig. 2B). Though, control of C_1 and C_5 cultivars yielded 2.2 and 1.77 t ha^{-1}, respectively; S_1 treatment of C_1 and C_5 cultivars produced, respectively 3.6 and 3.3 t ha^{-1} of seeds. All the treatments significantly increased the seed yield of C_1 cultivar. Only S_2, RS_2 and S_3 treatments increased the yield of C_5 cultivar by 85, 39 and 30%, respectively. S_2 was the most efficient treatment with 75% increment for C_1 and 85% for C_5.

DISCUSSION

The low biomass accumulation during 2004 cropping season may be attributed to the duration of solarization (4 weeks) that may have not been sufficient enough to induce higher biomass in C_1 cultivar.

Two months solarized plot increased the number of inflorescences for the two soybean cultivars. These results corroborate those of Braga *et al.* (2001) who observed that soil solarization generally stimulated plants fructification. The increase of flowers number of C_5 cultivar under rhizobial inoculation observed in this study was in agreement with findings of Okereke *et al.* (2001) who also reported enhanced flowering after inoculation of soybean varieties. Greater nodules number observed was in agreement with findings of Hungria *et al.* (2001) who obtained a significant increase of nodules number between 71 and 48.6% after inoculation of soybean with 15 strains of *Brady rhizobium*. Results on nodulation can be attributed to a substantial reduction of the number and activity of native rhizobia population, as well as other organisms like *Trichoderma* sp., *Actynomycetes*, mycorrhiza by soil solarization (Elmore *et al.*, 1997; Thuries *et al.*, 2000), which implies the need of reinoculation with more efficient strains.

Soil solarization improved the seed yield of the two soybean cultivars. Similar results were obtained by Banu *et al.* (1998) who reported enhanced rice yield by 25% with soil solarization and Mabong (2004) who observed 30% increased *Solanum tuberosum* yield after one month soil solarization. Increased plant growth response after soil solarization is a frequently observed phenomenon. This technique was also stated to promote the yield of several crops like tomato (Patterson, 1998), carrot, cabbage and faba bean (Mauromicale *et al.*, 2000), melon (Lira-Saldivar *et al.*, 2004). The main components of this effect may be seen in the control of soilborne pests and weeds and in the release of mineral nutrients from soil (Linke *et al.*, 1991). Solarization eliminates not only antagonistic microorganismis to rhizobia in the soil but it also renders inoculation more efficient and at the same time improves the physicochemical characteristics of soils. All these factors accounted for enhanced plant biomass, nodulation and seed yield in treatment RS_2 (Stapleton and DeVay, 1986).

Enhancement of yield by rhizobial inoculation corroborates with findings of Thao *et al.* (2002) who also related improved soybean yield to soil solarization. Soil temperatures achieved during two months soil solarisation were enough to inactive soilborne pathogens (McGovern *et al.*, 2002) and reduced the competitiveness of indigenous rhizobial population (Elmore *et al.*, 1997). In this study, reinoculation with more efficient rhizobial strains stimulated nodulation leading to biomass and yield enhancement of soybean. The improvement of yield obtained in this work is similar to 2.5 t ha^{-1} of soybean seed yield obtained by Schilling (2002) in USA after a hudge application of synthetic chemical.

Based on results obtained from this study, we can conclude that one month soil solarization was not sufficient to induce growth response of C_1 and C_5 soybean cultivars at Dang. Two months soil solarization increased plant biomass and seed yield. Seed inoculation with rhizobia improved nitrogen fixation through nodulation. The combined effect of two months soil solarization and rhizobial inoculation on soybean growth changed among cultivars. We intend to investigate on the effect of two months soil solarization on the control of parasitic weeds, soilborne pathogens and nutrient availability in the soil.

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