Dry Matter Accumulation and Physiological Indices of Two Soybean Cultivars in Response to Enriched Sewage Sludge Compost

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Abstract: Our objective was to examine the effect of organic amendments on dynamics of growth indices during the growth period in two new cultivars of soybean. The experiment was laid out in split plot based randomized complete block design with three replications in Mazandaran province, Iran, in 2006. Main plots were included 6 fertilizer treatments consisting 20 and 40 Mg ha⁻¹ sewage sludge (SS) compost and these levels of sewage sludge enriched with 50% recommended chemical fertilizers, chemical fertilizer treatment (50 Kg h⁻¹ urea and 75 Kg. h⁻¹ potassium sulfate and triple super phosphate) and control. Sub plots consisted of two genotypes of soybean (G32 and 033 promising lines). Dry matter accumulation, CGR, LAI and LAD were measured during the growth stages and plant characters such as number of pods, seeds and seed weight per plant and harvest index were determined. Results indicated that significant variations were found for dry matter, CGR, LAI and LAD between fertilizer treatments and soybean genotypes. The highest dry matter, CGR, LAI and LAD at different plant growth stages were obtained when 40 Mg ha⁻¹ enriched sewage sludge was applied. Analysis of plant growth parameters showed that the highest LAI at R₅ and LAD at R₅-R₆ belonged to soybean cultivar 033 in 20 and 40 Mg ha⁻¹ enriched sewage sludge. Accumulated dry matter and LAI peaked around R₅ or beginning seed and R₆ or physiological maturity, respectively. Maximum amounts of DMA, CGR, LAI and LAD during reproductive stages closely correlated to the higher dosage of enriched sewage sludge.

Key words: Soybean • Yield • Dry matter • CGR • LAI and LAD

INTRODUCTION

Seed yield of soybean [Glycine max (L.) Merr] has increased over the past century. The upward trend in on-farm yields is fueled by rapid producer adoption of technologies emerging from agricultural research, particularly improved adapted varieties [1]. Yield of a grain crop, however, is the product of interaction between genotypes and environment [2]. Donald [3] suggested breeding for yield improvement of a species in a particular type of environment by selection for physiological and morphological traits was thought to be conducive to high yields in that environment. Under given locations, plant characteristics such as vertical leaf orientation, high assimilatory capacity and high canopy reflectance, a short, stout stem conducive to maximum yield are of interest to soybean breeders [4-9]. Accordingly, there are two major pathways to ensure higher grain yield: increased harvest index and increased dry matter [1]. Modern cultivars had more dry matter production during seed filling period [10, 11]. On the other hands at recent years, composts are mostly used within agriculture as a source of N, P, Zn, Cu, Mn and organic matter. These soil amendments promote plant growth attributes when supplied with a balanced set of N, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn and B nutrients [12]. Perez-Murcia et al [13] were observed significant increases in the dry and fresh weights of broccoli aerial parts with increasing composted sewage sludge presence in the mixtures (peat + composted sewage sludge) . Similar effects of composts in growth media have been referred to by other researchers [15, 16]. Also, Guerrero et al., [17] had shown the feasibility of pine bark and sewage sludge mixtures as substitutes of peat in substrates formulation for growing Pinus pinea and Cupressus arizonica plants. Improvements to crop yield and reducing in chemical fertilizer usage, as seen following applications of green waste compost to forage maize [18], have obvious

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financial benefits and also reported that the plants growing in compost-amended plots had a reduced disease burden compared to un-amended plots. In order to have a better understanding of the physiological process of plant responses to soil amendments quantitative growth analysis can be used to make such comparisons and may provide important insights into the dynamics of crop, climate and soil interactions that will ultimately affect yield [19-21]. Effects of soil amendments such as sewage sludge on growth indices of soybean are not well investigated or documented especially in north of Iran conditions. The main purpose of this study was to evaluate the effect of sewage sludge compost enriched with chemical fertilizer on the dynamics of dry matter accumulation, crop growth rate (CGR), leaf area index (LAI) and leaf area duration (LAD) during the growth stages in two new soybean cultivars.

MATERIALS AND METHODS

This study was conducted in research farm of Sari Agricultural Science and Natural Resources University, Mazandaran Province, Iran (36° 39’ N, 53° 4’ E, altitude 16 m), in 2006. Soil and organic amendments characteristics at the beginning of the experiment are shown in Table 1. The experiment was laid out in a split plot based randomized complete block design with two factors and three replications. The plots were first ploughed in October, 2005 and then in opposite directions on May, 2006. The sewage sludge (SS) and chemical fertilizers (CF) were applied manually and mixed into the top 30 cm of soil a week prior to planting. Main plots had 6 fertilizer treatments as follows: T1 = 20 Mg ha⁻¹ SS, T2 = 40 Mg ha⁻¹ SS, T3 = 20 Mg ha⁻¹ SS + 50% CF, T4 = 40 Mg ha⁻¹ SS + 50% CF, T5 = CF (75 Kg ha⁻¹ potassium sulfate and triple super phosphate) based on soil test recommendations and T6 was kept as control without applying chemical or organic fertilizer and two genotypes of soybean (032 and 033 promising lines) consisted as sub plots. Those lines were semi determinate types and resistant to lodging and common diseases of soybean.

The plots consisted of five rows (3 meter long) with an inter-row spacing of 50 cm. The seeds were sown during May, 2006 and subsequently thinning was done to maintain 24 plants per m². Hand weeding helped to check weeds. Developmental stages of soybean were monitored weekly according to the staging system such as: flowering (R₁), full bloom (R₂), pod initiation (R₃), full pod (R₄), seed initiation (R₅), full seed (R₆) and physiological maturity (R₇) described by Fehr and Caviness [22].

Four plants from each plot were randomly selected to measure dry matter accumulation (DMA), crop growth rate (CGR), leaf area index (LAI) and leaf area duration (LAD) during the soybean growth stages. LAI was computed as the ratio between leaf area and the corresponding land area. DMA was obtained by oven drying the plant samples at 70°C until reaching a constant weight. LAD was calculated using the following equation:

\[ LAD = \frac{LAI_t + LAI_{t-1}}{t_f - t_i} \]  

Where LAI_t and LAI_{t−1} are the leaf area indices between two growth stages and t_i and t_f are the days corresponding to LAI determination.

Yield was obtained by removing 48 plants from the centre of 2m² in each plot. Harvesting was done manually and seeds were oven dried at 70°C until reaching a constant weight. Harvest index (HI) was calculated as the ratio between seed dry weight and total above ground dry weight at harvest. Data were analyzed using ANOVA (analysis of variance) and Duncan’s Multiple Range Test (DMRT) was used to separate means using SAS [23].

RESULTS AND DISCUSSION

Yield Characters

Seed Weight per Plant: The results of ANOVA (Table 2) indicated that main effects of SS and genotype were significant whereas there was no significant difference among interaction effects in terms of seed weight per plant. Application of 40 Mg ha⁻¹ SS + 50% chemical

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Texture</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Mn</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
<th>CN ratio</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Silty-Clay</td>
<td>0.23</td>
<td>0.14</td>
<td>278.95</td>
<td>13.96</td>
<td>5.57</td>
<td>1.02</td>
<td>58.47</td>
<td>10.95</td>
<td>7.52</td>
</tr>
<tr>
<td>SS</td>
<td>-</td>
<td>1.90</td>
<td>0.43</td>
<td>4893.9</td>
<td>235.64</td>
<td>57.00</td>
<td>471.49</td>
<td>16542.11</td>
<td>7.8</td>
<td>7.44</td>
</tr>
</tbody>
</table>
fertilizer (T₃) to the soybean plots increased the soybean seed weight compared to chemical fertilizer and other organic fertilizers plots. Also, T₁ (40 Mg ha⁻¹ SS), T₃ (20 Mg ha⁻¹ SS + 50% chemical fertilizer) and chemical fertilizer increased seed weight (g/plant) more than T₁ (20 Mg ha⁻¹ SS) and control treatments. The lowest amount of this trait was obtained from the control plants (Table 2). Mehmet et al., [24] reported that the highest seed weight in Lentil (Lens culinaris Medc.) was determined from 60 Mg ha⁻¹ sewage biosolid applications. Similarly, Mekki and Ahmed [25] revealed that the biofertilizer treatment alone, increased seeds weight of soybean plant. In another research, Tung and Pamela [26] revealed that soybean seed yield from biodynamic, organic or chemical production practically the same and significantly higher by 50-69% than that of the control. In this experiment, seed weight per plant was highest in 033 cultivar (Table 2).

**Pod Number per Plant:** The pod number per plant for different treatments is summarized in Table 2. These data showed significant differences between fertilizer treatments and cultivars in terms of this character. The highest number of pod per plant was recorded in T₁ (40 Mg ha⁻¹ enriched with chemical fertilizer). CF, T₁, T₃ and T₅ treatments effects on number of pod per plant were similar (Table 2). In agreement with this, recent finding have shown that application of organic manure + yeast as one treatment caused the maximum seed weight and number of pods per plant, seeds number per pod and 1000-seed of soybean plant [25]. Likewise, Tawadeh et al., [27] revealed that the highest number of pods/plant of soybean was for the chemical fertilizer (41.2 pods) and the soil mixed with sewage sludge, 5% (w/w) (40.2 pods) plots, while the 0, 15 and 20% mixture had lowest number (26.2, 28.0 and 22.0 pods, respectively). Between soybean cultivars, maximum number of pod was produced in 033 cultivar. Similar results were obtained for grain yield per hectare (data not shown).

**Seed Number per Plant:** Both chemical fertilizer and organic amendment treatments affected numbers of seed per plant as well as pod number per plant significantly. Growing soybean in the organic amendment plots except T₁ showed the higher seed number per plant than chemical fertilizer. The maximum number of seed per plant (189.64) was obtained from 40 Mg ha⁻¹ SS enriched with chemical fertilizer treatment and the lowest number of seed per plant (87.05) was obtained from the control plant (Table 2). In concurrence with this, the maximum seed number per lentil plant was found from 60 Mg ha⁻¹ sewage biosolid [24]. The data from table 2 showed that the highest seed number per soybean plant belonged to 033 cultivar.
Harvest Index (HI): Results showed that 20 and 40 Mg.ha\(^{-1}\) of sewage sludge enriched with chemical fertilizers produced highest harvest index (28.6 and 28.1\%, respectively) and the control treatment showed the lowest value (18.8\%). Meanwhile, the 20 Mg.ha\(^{-1}\) of sewage sludge and chemical fertilizers treatment produced higher harvest index compared to the 40 Mg.ha\(^{-1}\) of sewage sludge treatment (Table 2). While reports claim that harvest index in corn plant were unaffected by manure treatments [28]. There was a significant correlation between soybean grain yield and seed weight (g/plant) (r=0.90\**, pod number per plant (r=0.95\**), seed number per plant (r=0.81\**) and harvest index (r=0.77\**, data not shown).

Growth Indices

Dry Matter Accumulation (DMA): The dynamics of dry matter accumulation varied among fertilizer treatments. Dry matter in genotypes peaked around R\(_5\) (beginning maturity) stage. 40 Mg.ha\(^{-1}\) enriched with SS caused maximum dry matter accumulation in soybean genotypes at all the growth stages. Furthermore, application of T\(_1\), T\(_2\), T\(_3\) and chemical fertilizer treatments caused similar increases in dry matter accumulation during reproductive stages except R\(_5\) (Fig 1-a). Mean comparisons showed that interaction effects of fertilizer and cultivar had significant differences on plant dry matter accumulation at 41 and 55 days after sowing, so that the maximum dry matter at this time were observed in 033 line when 20 and 40 Mg.ha\(^{-1}\) enriched SS was applied (data not shown). Growing of corn in composted-manure-treated soils produced 12% greater aboveground DM in 2000 and 15% greater DM in 2001 compared to fresh manure treated soils [28]. Results indicated a close relationship between accumulative dry matter at reproductive stages and seed yield (r = 0.92\* to 0.98\*). Similarly, dry matter of soybean accumulation at R\(_3\), R\(_4\), R\(_5\) and R\(_6\) stages markedly differed, showing the highest value for the 5\% mixture and the lowest value for the 20\% mixture. Also, increasing of sewage sludge amount significantly decreased in dry matter for reasons same as for the number of soybean nodes [27]. Also, dry matter of Chinese cabbage decreased when these were planted under high conditions of lead and zinc (more than 10.0 and 15.0 mg/kg, respectively) [29]. With regard to different soybean cultivars, the 033 cultivar produced significantly higher dry matter accumulation compared to 032 at the growth stages. Dry matter in 033 was accumulated average 15.90\% more than 032 at R\(_6\) (Fig. 1-b). The total dry matter accumulation appears to be a more important contributor to yield improvement [30]. In addition, the accumulation of dry matter from R\(_5\) stage is an important factor in attaining high yields [31]. Significant higher dry matter in new varieties after pod filling was also reported [32].

Table 3: The correlation coefficients of seed yield with leaf area index (LAI), dry matter accumulation (DMA) and crop growth rate (CGR) in different fertilizer amounts and cultivars (n=18)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Developmental stage</th>
<th>032</th>
<th>033</th>
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<tr>
<td>LAI</td>
<td>V5</td>
<td>0.95**</td>
<td>0.82**</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.96**</td>
<td>0.85**</td>
</tr>
<tr>
<td></td>
<td>R4</td>
<td>0.95**</td>
<td>0.85**</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>0.96**</td>
<td>0.89**</td>
</tr>
<tr>
<td>DMA</td>
<td>V5</td>
<td>0.77**</td>
<td>0.98**</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.94**</td>
<td>0.97**</td>
</tr>
<tr>
<td></td>
<td>R4</td>
<td>0.96**</td>
<td>0.98**</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>0.96**</td>
<td>0.97**</td>
</tr>
<tr>
<td>CGR</td>
<td>V5</td>
<td>0.82**</td>
<td>0.95**</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>0.96**</td>
<td>0.96**</td>
</tr>
<tr>
<td></td>
<td>R4</td>
<td>0.96**</td>
<td>0.95**</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>0.74**</td>
<td>0.94**</td>
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</table>

\* Statistical difference at P< 0.01

Crop Growth Rate (CGR): Similar to DMA, CGR of two soybean cultivars was significantly influenced by different fertilizer treatments throughout their growth stages so that 40 Mg.ha\(^{-1}\) enriched sewage sludge caused the maximum CGR. Analysis results indicated that T\(_1\) (20 Mg.ha\(^{-1}\) SS), T\(_2\) (40 Mg.ha\(^{-1}\) SS), T\(_3\) (20 Mg.ha\(^{-1}\) enriched SS) significantly affected on soybean crop growth rate similar to T\(_4\) (chemical fertilizer alone) treatments (Fig. 2-a). Composted manure produced a greater (2.0 vs. 1.8 g. m\(^{-2}\) GDD\(^{-1}\)) and slightly later (880 vs. 820 GDD) maximum CGR of corn than did the fresh manure, leading to greater late-season biomass production [28]. This greater late-season biomass production in the composted manure was maintained through longer leaf area duration, as indicated by the treatment differences in LAI at the last measurement date. Also between soybean cultivars, 033 had higher CGR than 032 at all of its growth stages (Fig 2-b). Mean comparisons showed that interaction effects of fertilizer
Dry matter accumulation (g. m\(^{-2}\))

Fig. 1(a,b): Dynamics of dry matter during growth stages in different fertilizer amounts and in soybean genotypes. An ellipse is drawn around fertilizer amendments with similar dry matte indices and fertilizer amendments not included within an ellipse are different (**P< 0.01) at that sampling time. Vertical bars represent ± standard error of the mean.

**T1** = 20 Mg.ha\(^{-1}\) SS, **T2** = 40 Mg.ha\(^{-1}\) SS, **T3** = 20 Mg.ha\(^{-1}\) SS + 50% chemical fertilizer, **T4** = 40 Mg.ha\(^{-1}\) SS + 50% chemical fertilizer, **T5** = chemical fertilizer (potassium sulphate and triple superphosphate (75 Kg.ha\(^{-1}\)) based on soil test recommendations and **T6** = control (no chemical or organic fertilizer application)
Fig. 2(a,b): Dynamics of CGR during growth stages in different fertilizer amounts and in soybean genotypes. An ellipse is drawn around fertilizer amendments with similar crop growth rate indices and fertilizer amendments not included within an ellipse are different ($P=0.05$ or $**P=0.01$) at that sampling time. Vertical bars represent ± standard error of the mean.
Fig. 3(a,b): Dynamics of LAI during growth stages in different fertilizer amounts and in soybean genotypes. An ellipse is drawn around fertilizer amendments with similar leaf area index indices and fertilizer amendments not included within an ellipse are different (*P= 0.01) at that sampling time. Vertical bars represent ± standard error of the mean.
Fig. 4 (a,b): Dynamics of LAD during growth stages in different fertilizer amounts and in soybean genotypes. An ellipse is drawn around fertilizer amendments with similar leaf area duration indices and fertilizer amendments not included within an ellipse are different (*) at that sampling time. Vertical bars represent ± standard error of the mean.
and cultivar had significant differences at during R1 - R2 and R2 - R3 stages so that the maximum DM was observed in 033 cultivars with 20 and 40 Mg ha⁻¹ enriched sewage sludge amendment. Correlation analysis revealed that values of CGR during reproductive stages were positively correlated to grain yield (r = 0.76" to 0.96", data not shown). In our study, seed yield was positively and strongly correlated with CGR (V2) (r = 0.82" and r = 0.95"), CGR (R2) (r = 0.96" and r = 0.96"), CGR (R2) (r = 0.96" and r = 0.95") and CGR (R3) (r = 0.74" and r = 0.94") in 032 and 033 lines, respectively (Table 3).

**Leaf Area Index (LAI):** Leaf area index of soybeans (P= 0.01) was significantly affected in all fertilizer treatments. At the reproductive growth stage of R1 (beginning bloom; 2.89), R2 (full bloom; 3.86), R3 (beginning pod; 4.72), R4 (full pod; 5.69) and R5 (beginning seed; 8.00) the 40 Mg ha⁻¹ sewage sludge enriched with chemical fertilizer treatment markedly increased LAI, however, no significant differences were detected among T2, T3 and chemical fertilizers alone at the growth period. LAI peaked around R2 stage and then declined gradually (Fig 3-a). Development of LAI varied among soybean genotypes. The 033 line had the highest LAI from R1 to R3 sampling dates (Fig 3-b). Higher corn yields obtained from compost were achieved by greater LAI and leaf area duration [28]. Analysis of plant growth parameters showed that the highest soybean LAI belonged to 033 cultivar in 20 and 40 Mg ha⁻¹ enriched sewage sludge at the growth stages (data not shown). These results are consistent with those of Bachman and Metzger [33] who revealed that growth increases up to 40% were observed in dry shoot tissue and leaf area of marigold, tomato, green pepper and corn in vermicompost treatments compared to control plot.

In our experiment, there were strong positive correlations between DMA, CGR, LAI, LAD and soybean grain yield (data not shown). Some researchers suggested that soybean yield positively related to LAI and dry matter at the R2 stage [30, 34, 35]. Accordingly, accurate simulation of leaf area index is necessary for the predicted simulation of biomass accumulation and transpiration. The opposite is also true, partitioning to the leaves to form new leaf area influenced by biomass accumulation. A typical LAI pattern begins with a lag increase early in the season, followed by a rapid increase of LAI until a maximum value is reached, then a decline of LAI as leaves senesce and plants reaches physiological maturity, for example: Setiyono et al., [36]. Significant and positive relationships between seed yield and LAI in 032 and 033 lines, (r = 0.95" to r = 0.98" and r = 0.82” to r = 0.89”, respectively) at the V3 (55 days after planting), R2, R3, R4 and R5 stages (Table 3).

**Leaf Area Duration (LAD):** Comparison of LAD changes curves indicated that among fertilizers treatments, 40 Mg ha⁻¹ enriched sewage sludge and between two genotypes, the 033 cultivar had maximum value of LAD at the growth process (Fig 4-a-b). The results of ANOVA showed that interaction effects of fertilizer and cultivar had significant differences on LAD in soybean cultivars so that the maximum value of LAD was obtained when the 20 and 40 Mg ha⁻¹ enriched sewage sludge for 033 cultivar was used at all the growth stages. The values of LAD at R2-R3, R3-R4, R4-R5 and R5 stages were strongly and significantly correlated to grain yield (r = 0.92", 0.92", 0.90" and 0.93" respectively, data not shown).

**CONCLUSION**

Leaf area index (LAI), leaf area duration (LAD) and dry matter accumulation (DMA) strongly influence on yield components of soybean during the reproductive period and the results showed the importance of these growth indices to soybean performance. In our experiment, the maximum DMA, CGR, LAI and LAD at the growth process were observed in the highest dosage of sewage sludge in combination with chemical fertilizer, probably increases readily available nutrients of soybean plants resulted in accumulation of more assimilates. Other organic amendments produced DMA, CGR, LAI and LAD similar to chemical fertilizer. Accumulated of dry matter and LAI peaked around R2 and R3, respectively. In the current study, the 033 line was superior over 032 and produced maximum value of DMA, CGR, harvest index (HI), number of pod, seed per plant and seed weight and also produced maximum LAI and LAD at the growth stages when the 40 Mg ha⁻¹ enriched sewage sludge was applied.

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