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Full Length Research Paper

Propagation studies in *Gloriosa superba*

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Studies on seed germination in glory lily revealed that seeds soaked in hot water for one hour results in maximum germination of 32.75%, with earlier germination (48.35 days) and vigor index (565.92), when compared to other chemical treatments or growth regulator. Effect of certain growth regulators (GA_3, Kinetin, Ethrel) on the sprouting of tuber was assessed and ethrel at 500 ppm recorded maximum sprouting percentage (100 %), earlier sprouting of tubers (6.33 days), maximum plant height (99.32 cm) and maximum number of leaves per plant (34.04) and plant girth (1.81 cm).

Key words: Glory lily, seed treatment, growth regulator, tuber, germination, sprouting.

INTRODUCTION

Gloriosa superba L. (Colchicaceae) is an important medicinal plant, native to Tropical Asia and Africa. It is highly valued in modern medicine due to the presence of Colchicine and Colchicoside which are used in treatment of gout and rheumatism (Farooqi et al., 1993). It is commercially propagated by 'V' or 'L' shaped tubers, sourced from the wild especially from the forest areas and hillocks. It is distributed throughout India and commercially cultivated in Tamil Nadu (Saxena and Brahmam, 1995). It has been reported that more than 500 tonnes of wild tubers are collected every year and used for planting in Tamil Nadu alone. About 800 kg of tubers are required to plant in one acre. The cost involved towards planting material (Rs.250 to 300/kg of tubers), alone accounts to 2.0 lakhs at Rs 250 per kg of tuber prevailing for the last three years. Seed germination is erratic and takes three weeks to three months (Azhar and Sreeramu, 2004). But the sprouting of tubers is irregular and in a period of 30 days they sprout to an extent of 60%. Hence this experiment was aimed at standardizing the seed treatment methods and the effect of growth regulators on sprouting of tubers of *G. superba.*

MATERIALS AND METHODS

Dried seeds of *G. superba* collected from Mulanur of Tirupur district were used for standardizing the seed treatment. To test the effect of hot water soaking on seed germination, 250 ml of water was heated up to 100ºC and then taken away from the heat source. The seeds were immersed in hot water for an hour and then soaked in cold water for 12 h. Similarly, seeds were soaked in chemicals *viz.,* GA_3 (100 and 250 ppm), Thiourea and Potassium nitrate at various concentrations (0.5, 1.0, and 1.5%) for an hour. Treated seeds were sown in the raised beds at a distance of 10 cm between the lines in the beds. Seeds of 100 numbers were sown in each treatment with the replication of four.

To standardize the effect of growth regulator on sprouting, healthy tubers of *G. superba* were collected from farmer's field, Mulanur of Tirupur district. These tubers were soaked in different growth regulators viz., Kinetin (25 and 50 ppm), GA_3 (100 and 200 ppm) and Ethrel (250 and 500 ppm) for one hour. Tubers were planted in the medium sized pot filled with pot mixtures containing sand, red earth and FYM (1:2:1). A completely randomized design with three replications was used, while the dependent variables

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Table 1. Effect of seed treatments on seedling characters of *Gloriosa superba*.

Seed treatment (one hour soaking)	Germination percentage (%)	Days for germination (days)	Shoot length (cm)	Root length (cm)	Vigour index
Control	0.00 (0.45)	0.00	0.00	0.00	0.00
Hot water soaking	32.75(34.88)	48.35	11.12	6.15	565.92
Thiourea - 0.5%	11.00(19.17)	52.95	10.51	5.48	175.94
Thiourea - 1.0%	7.50(15.88)	57.55	10.68	5.80	123.67
Thiourea -1.5%	8.00(16.16)	60.80	10.76	5.78	132.32
KNO_3 - 0.5%	13.75(21.72)	55.00	10.62	5.78	225.63
KNO_3 - 1.0%	16.25(23.57)	56.75	10.65	5.63	264.71
KNO_3 - 1.5%	15.25(22.75)	49.80	10.84	5.43	248.27
GA_3 - 100 ppm	12.20(20.47)	50.15	11.05	6.45	214.43
GA_3 - 250 ppm	19.50(24.93)	48.45	11.78	6.94	365.23
Mean	13.47(20.00)	47.99	9.80	5.34	231.42
SE(d)	1.9905	0.9920	0.2186	0.1959	40.7236
CD(0.05)	4.0842	2.0356	0.4486	0.4020	83.5600

Values in parenthesis are arcsine transformed.

were measured for two months after planting.

RESULTS AND DISCUSSION

Dormancy is a condition where seeds will not germinate even when the environmental conditions (water, temperature and aeration) are favourable for germination. Poor and delayed seed germination in *G. superba* was reported and the germination was erratic which took three weeks to three months (Azhar and Sreeramu, 2004). The water impervious seed coat protects the plant from germination during the harsh condition until the rainy season. In the present study, various dormancy breaking treatments revealed that hot water treatment imposed for an hour recorded the higher germination percentage (32.75%), earlier germination (48.35 days), and vigor index (565.92) (Table 1). Soaking of seeds in hot water could have helped in enhancing the seed germination by softening the hard seed coat and facilitating leaching out of the germination inhibitors. Similar increase in germination consequent to hot water soaking has been earlier reported in *Sesbania rostrata* (Sarker et al., 2000), *Tephrosia purpurea* and *Abrus precatorius* (Singh et al., 1984). Hot water treatments have been reported to enhance germination of hard coated seeds by elevating water and O_2 permeability of the testa (Aydin and Uzun, 2001).

Other treatments such as thiourea, KNO_3, GA_3 also increase the germination percentage, it was not as high as hot water treatment. This implies that hard seed coat is the prime factor for seed dormancy in glory lily. Further, hot water soaking treatments also enhanced the quality of seedlings as evidenced by higher shoot length, root length, vigour index besides recording lesser days for germination. Hence, this practice can be recommended to raise the nursery.

Treatment with growth regulators and other chemicals on induction of better seed germination as compared to control where no germination was recorded might be due to the antagonistic effect on growth inhibitors and also enhancement of the rate of metabolism during germination (Verma and Tondon, 1988). Thus, among all the seed treatments, soaking the seeds in hot water for one hour was the most effective for inducing better germination of seeds in Glory lily.

G. superba is propagated mainly during the rainy season (June-July) by V-shaped tubers. Vegetative propagation is very slow as the maximum number of daughter tubers produced per year is two. Sprouting of the tubers is irregular and reaches about 60% in 30 days (Krause, 1988).

The effect of various growth regulators (GA_3, kinetin, ethrel) on sprouting of tubers were studied (Table 2). It was found that ethrel at 500 ppm gave the highest sprouting percentage (100%) and days for sprouting (6.33 days). Similar reports

Table 2. Effect of growth regulators on plant characters of *Gloriosa superba.*

Treatment	Sprouting percentage (%)	Days for sprouting (days)	Plant height (cm)	No. of leaves per plant	Stem girth (cm)
Kinetin 25 ppm	60.00(50.76)	7.22	39.11	24.5	0.93
Kinetin 50 ppm	73.33(59.20)	9.66	27.65	13.53	0.80
GA_3 100 ppm	80.00(67.85)	7.44	59.86	28.11	0.97
GA_3 200 ppm	86.66(72.07)	6.66	86.22	33.88	1.35
Ethrel 250 ppm	66.66(59.78)	6.88	56.87	26.11	0.57
Ethrel 500 ppm	100(89.37)	6.33	99.32	34.04	1.81
Control	53.33(46.91)	10.44	21.57	27.10	0.56
Mean	74.28(63.717)	7.8071	55.8019	27.74	0.99
SE(d)	11.5436	0.5483	20.4899	6.4551	0.3454
CD (0.05)	24.7613	1.1761	43.9513	13.8464	0.7408

Values in parenthesis are arcsine transformed.

were earlier reported in Gloriosa (Rajaram et al., 2002; Suh, 1989; Puja et al., 2003).

Treatment of tubers with GA_3 200 ppm recorded higher sprouting rate of 86.66%, with earliness in sprouting (6.66 days). According to Groot and Karssen (1987), gibberellins either endogenous (or) exogenous was considered to be an important factor in inducing sprouting. The effect of gibberillic acid in inducing the formation of hydrolytic enzymes may be a factor which might have regulated the mobilization of reserves, ultimately resulting in early sprouting with GA_3. This is also in close conformity with reports of Bhattacharjee et al. (1994) who reported that GA_3 (10-100 ppm) at all concentrations recorded earlier sprouting of bulbs of tuberose.

The present investigation also shows that ethrel 500 ppm significantly increased the plant height, number of leaves and girth of the plant followed by GA_3 at 200 and 100 ppm. Similar findings were given by Puja (1999), who reported that tubers treated with 500 ppm ethrel gave better vegetative growth and tuber yield. Increased vegetative growth and tuber yield were observed in *G. superba* due to treatment with ethrel at 500 ppm. The results are in accordance with the findings of Jayachandran and Sethumadhavan (1979) in ginger who reported that 200 ppm of ethrel resulted in maximum leaf production.

The increased plant height recorded by GA_3 200 ppm in the present study might be due to its role in cell division and cell enlargement and are largely controlled by endogenous level of gibberellic acid which has been proved in number of crops. The increased cell division and cell elongation reflected in increased plant height was observed in hybrid lilies (Hanks and Menhennett, 1980).

Tallest plants with more number of leaves were produced in gladiolus when the corms were treated with 300 ppm GA_3 as reported by Rajesh and Ajaykumar (2007). Similar results were obtained with GA_3 in day lily (Das et al., 1992), *Lilium longiflorum* (Sujatha and Bhattacharjee, 1992), gladiolus (Bhattacharjee, 1984) and in Zephyranthes (Sujatha and Bhattacharjee, 1990).

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Rural women's access to productive resources: Implications for poverty reduction- the case of Gamo Gofa Zone, Southern Nations, Nationalities, and Peoples' Region (SNNPR)

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Rural areas of Ethiopia strikingly vary in terms of social and economic structure, geography and culture. The same is true for rural women's participation in productive activities. Likewise rural women of Gamo Gofa Zone are experiencing inadequate right to access different productive resources like land and other resources they require. This study therefore, intends to identify the existing rural women's access to productive resources and its effect on the stated poverty reduction strategy in the study area. The study explicitly reflect as the rural agriculture sector, where gender inequalities in access to productive resources are persistent, undermining a sustainable and inclusive development of the sector and their contribution for poverty reduction.

Key words: Rural women, access to, domestic chores, division of labor, poverty reduction.

INTRODUCTION

Back ground of the study

Rural areas of Ethiopia strikingly vary in terms of social and economic structure, geography and culture. Likewise rural women are not homogeneous groups. They have different roles and occupations, on farms and in family businesses, in employment and in community activities. Their needs and interests differ too, particularly from one age group to another, and depending on the size and composition of their family and age of their children. The economic and social changes that rural areas are undergoing do not affect all women in the same way. Offering opportunities to some, to others they bring difficult challenges (Akuna, 2004). Despite of these differences almost all Ethiopian women perform multiple roles for the survival of their homes and the nation. In addition to this there are inequalities in the distribution of food, health care, employment opportunities (World Bank, 2001, 2007). Overall evidence suggests that the scale of poverty in the developing countries continue to worsen despite investments in poverty reduction (Jazairy et al., 1992). The tasks which the rural women are expected to perform and the skills needed to carry them out vary. Men hold superior position in households and

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Table 1. Gender and division of labor.

S/N		Mothers		Fathers		Both		Neither	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1	Girls have to help	142	78.9	1	0.6	37	20.6	0	0
2	Boys have to help	21	11.7	114	63.3	31	17.2	14	7.8
3	Who sleeps more	2	1.1	175	97.2	0	0	3	1.7
4	Who works more in house	177	97.3	0	0	0	0	3	1.7

Source: Field survey.

communities and women are put to inferior position (NAP-GE, 2006). In women's access to productive resource issues power in gender relation implies men's higher access to productive resource and women's less access productive resource. Rural women's access to productive resources has become more critical in developing countries like in Ethiopia as productive resources are the means to alleviate poverty and bring sustainable rural development (Tesfaye, 2003). These conditions necessitate the assessment of rural women's access to productive resources and its implication in Gamo Gofa Zone.

Access to productive resources

Resources are means and goods including those that are economic like household income, productive like land, equipments, agricultural inputs (including labor) and opportunity to leadership and decision-making, information, organization and time. Access to resource implies to the ability to use resources and/or benefits and to make short-term decisions on these resources (Sida, 2003). This study therefore, intends to identify the labor division in the area: Find out the existing rural women's access to productive resources and its effect on the stated poverty reduction strategy in Gamo Gofa Zone of SNNP Region.

METHODOLOGY

There are 15 woredas (Districts) in the Gamo Gofa Zone with different agro-ecologies. This study carried out in two woredas from different agro-ecology that have been selected by simple random sampling technique. Three kebeles was selected from each two woredas by using lottery method of random sampling and then totally six kebeles (the lowest government administration structure) were selected to represent the zone. Finally, for survey questionnaire 30 respondents per kebele was selected using simple random sampling technique. Totally, 180 rural women were participated to respond the interview questionnaire of the study. Whereas 5 women and 5 men were selected for group discussion at each woreda based on their nature to act as the opinion leader. Both quantitative and qualitative data are used for this study. Structured interview questionnaire used for quantitative data and Semi-structured interview questionnaire are used for qualitative data. The interview was conducted at appropriate time they want to be interview.

The study mainly focuses on gender and division of labor and the access of rural women's to productive resources. Gender and division of labor contain the variables like who work more, whom boys and girls help more and who sleeps more. Gender based distribution of household activities includes the variables: Cooking, fetching water, collect fire wood, cleaning work, washing clothe, cares the children, take care of patient, grinding grains and purchasing salt and others. Land, labor, water, livestock, inputs, and finance are the variables included for assessing the rural women's access to productive resources.

To get background information and the number of the study area, secondary sources were reviewed. The primary data were obtained from the rural women and men by using appropriate data collection instruments like questionnaire, focus group discussion, and interview and field observation. Data was analyzed using the Statistical Package for the Social Science (SPSS) and some descriptive statistics, such as percentage and mean.

RESULTS AND DISCUSSION

Gender based division of labor

Gender division of labor in households is the main economic strategy used to meet family basic needs for shelter, food, health, procreation and education. And yet, the nature of this division of labor is one that constrains development. A number of factors are responsible for the gender division of labor today: Some are gender-neutral and others are gender-biased. The facts are shown in the following Table 1. As indicated in Table 1, girls help (about 78.9%) her mothers. Girls help their mother than boys in working domestic chores and boys have to help their father. In another way mothers discharge about 97.3% of domestic chores. Hence, if girls are expected to help their mother, both of them are expected to play reproductive role which is categorized under unpaid work. This elucidated as almost all domestic chores are on the shoulder of rural women. The respondents assured as fathers sleep more (97.2%) than mothers.

Discussants come in to consensus as rural women do all of the domestic chores, working on average up to 16 h a day in most of rural houses while conducting group discussion. This data is in line with the most literature report as rural women works on average 16 h in developing countries (Braunstein, 2008; Elson, 2009;

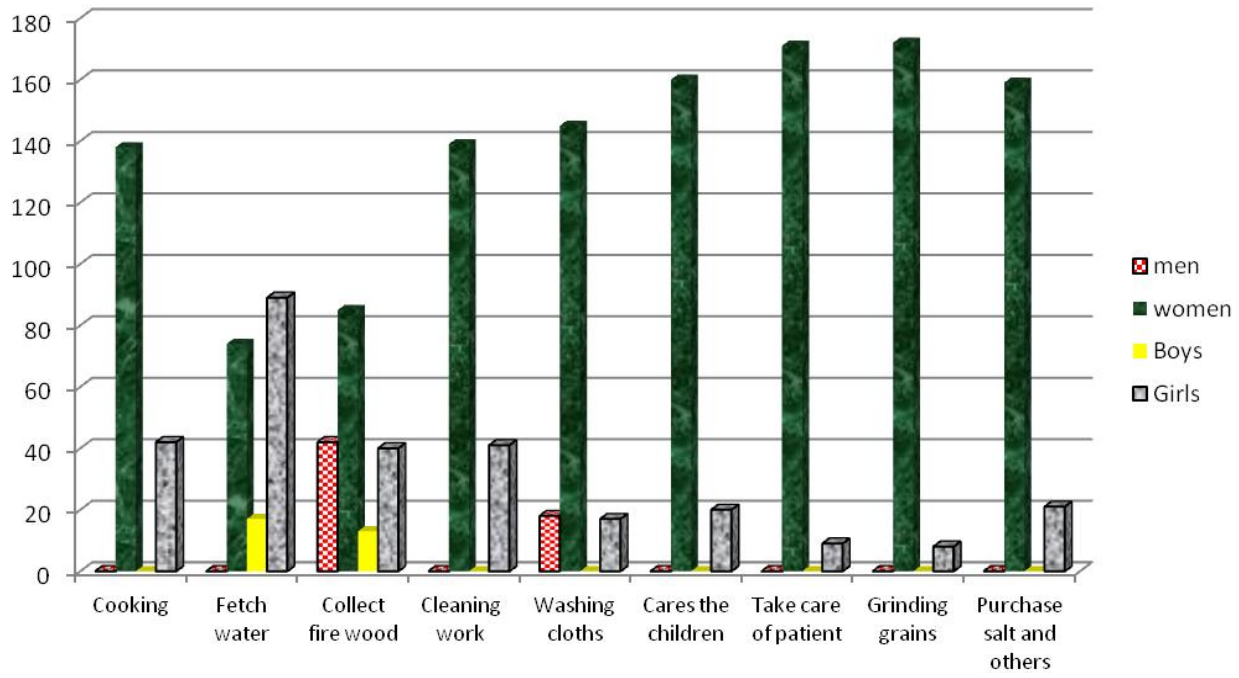

Figure 1. Gender based distribution of household activities.

FAO, 2011; NAP-GE, 2006).

The domestic chores described further and main activities are identified in group discussion to interview as whose responsibility are they. According to this survey result the number of hours men commit to housework has remained roughly none in the study area. While women west her working hour's almost in home work and related role. Figure 1 shows that rural women's are an implementer of these main unpaid domestic activities'. Figure 1 explicit as women dominate or almost sole contributor on activities like cares the children (88.9%), takes care of patient (95%), grinding grains (95.6%), purchase salt and other (88.3%) and shares the activities like fetching water, food preparation and cleanup with her daughters. The share of men is restricted only on activities like collecting fire wood (23%) and washing clothes (10%). Mainly these activities are carried out when the woman are pregnant and gives birth. The above facts give more sense when it presented in the form of histogram. It helps to compare the contribution of household members in main domestic activities.

As it is displayed on the above graph except fetching water where girls take the great share other domestic chores are done by women. Girls are participating in each and every domestic role even though their role is less than women. Whereas men has a role in two domestic activities and boys share water fetching duty with girls and women.

The above pie chart displays the fact of labor division related to domestic chores on an average, 77% of women, 18% of girls**,** 3% of men and 2% of boys. Women in rural areas generally bear primary responsibility for the nutrition of their children, from gestation through weaning and throughout the critical period of growth. In addition, they are the principal food producers and preparers for the rest of the family. Women play their domestic role while they are participating at significant level in different crop production activities or farm works. Despite their contributions to food security, women tend to be invisible actors in development. All too often, their work is not recorded in statistics or recognized by society or mentioned in reports. As a result, their contribution is poorly understood and often underestimated. The data found in Figure 2 reveals this fact. There are many reasons for this. Work in the household is often considered to be part of a woman's duties as wife and mother, rather than an occupation to be accounted for both men and women.

A great deal of rural women labor - whether regular or seasonal – goes unpaid and is, therefore, rarely taken into account in official statistics (UN ECA, 2004). If women were more likely to be breadwinners, they had have less to do around the house or domestic chores should be equal responsibility for both partners (World Bank, 2001).

Rural women's access to productive resources

Improved access to productive resources like land and credit and other will facilitate the opening up of new opportunities for rural women, which in turn will provide

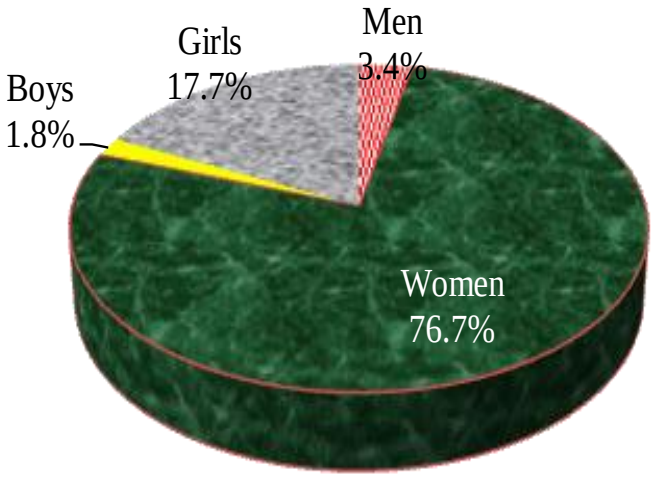

Figure 2. Average shares of domestic activities.

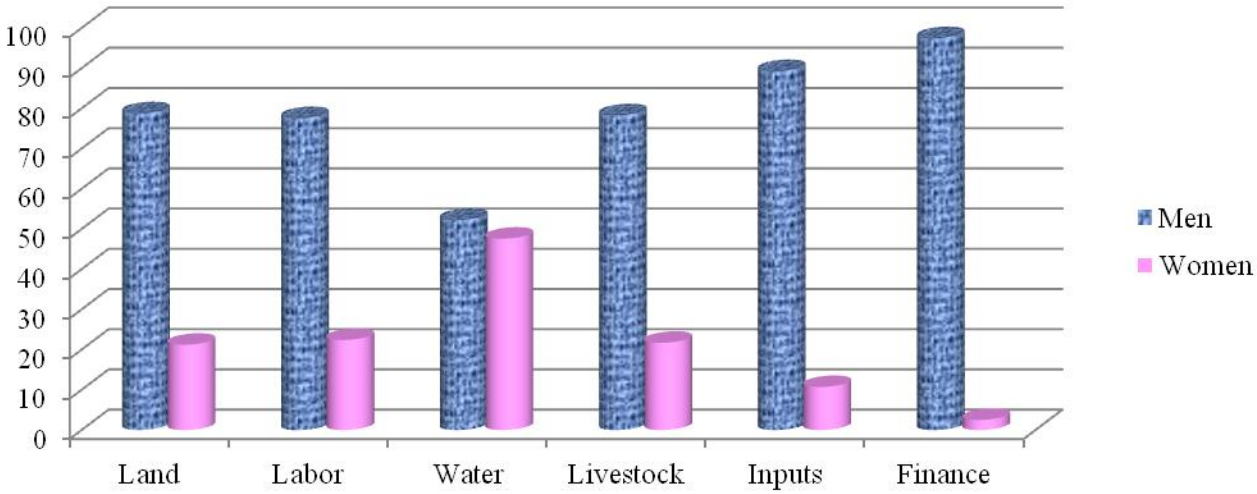

Figure 3. Access to productive resources.

even further stimulus to rural economic growth and well-being. As illustrated in Figure 3 the results of the data analysis show limited access by female farmers to key productive resources and agricultural services. Females in both male headed households and female-headed households tend to have poor access to productive resources because of gender division of labor and cultural barriers. The male farmers enjoy better access to productive resources mainly because of the culture, family headship and gender division of labor.

As illustrated here, the results of the data analysis show limited access by female farmers to key productive resources and agricultural services. Females in both male headed households and female-headed households tend to have poor access to productive resources because of gender division of labor and cultural barriers.

The male farmers enjoy better access to productive resources mainly because of the culture, family headship and gender division of labor.

Access to land

As to data above, men have 78.81% of access to land where women have only 21.19% of access land. This is an indicator how much they are neglected from crucial resource in rural agrarian community's productive resource. The access to land is decisive position in rural community.

Land lessens is a global phenomenon that disproportionately affects women. The rights of women to own, use, access, transfer, inherit and otherwise take decisions about land are recognized throughout the research area.

Not only do women have less access to land than men, but they are also often restricted to so-called secondary land rights, meaning that they hold these rights through male family members, and thus she is under the risk of losing these entitlements in case of divorce, widowhood or the migration of the male relative. Frequently, women have only user rights, mediated by men, and those rights remain highly precarious. Rural women have much less or insufficient access to land, membership in rural organizations, credit, agricultural inputs and technology, training and extension services, and marketing services.

Labor

Table 1 reveals that men have 77.58% of access to productive resources and women have 22.43% of access to productive resources. Women dominantly have a full access (98.9%) to girls' labor. Other type of labor is hardly accessed by rural women.

Water

Rural women tend to have high access to water than their counter part. But the reality is very different. Women have 47.54% of access to water and men have 52.34% of access to water resources. When the access to different sources of water is compared women have better access; 84.4 and 97.8% of access consecutively in potable water and sanitation water. Whereas, their access to livestock water and spring water is very less than men's access to it.

Livestock

According to the data from Table 1, 78.35% access to livestock is for men and only 21.66% of access is for women. When one looks separately the access of rural women for cow is about 84.4% and for poultry is about 84.5%. Their counterpart have less access for these both type of livestock. In opposition to this the access of men to another type of livestock is almost 100% and women have no access to these types of livestock.

Inputs

The access of rural women for different type of inputs is 10.7 and 89.3% for men. When we look separately they have only access to poultry related inputs. The access of women farmers to agricultural inputs and technologies is constrained by their lack of access to credit and membership in rural organizations, but also by gender blind development programs and lack of attention to the needs of women farmers in research and technology development programs.

Because women farmers everywhere are engaged in a wide range of laborious tasks related to food security, there is a need for the development and introduction of appropriate laborsaving technology in food processing and storage as well in food production, and in related areas such water, sanitation, fuel and food preparation.

Finance

Surprisingly rural women have only 2.5% of access for different type of financial resources and men have 97.5% of access to men. This is a sign as rural women have no access to different financial resources, if they fail to have enough access to financial resources their ability to participate in any income generating activities will be almost null. This is a serious obstacle to improving women's agricultural productivity, as without credit rural women are unable to buy inputs such as seeds, fertilizers, and improved technologies, or to hire labor. Paradoxically, numerous studies have shown that women are more likely than men to repay loans.

Because rural man and rural women often have different responsibilities in agricultural production and food security, both need credit according to their needs. It is thus important for rural women to have not only access to credit but also control over the use of the credit so that it is not diverted to male-dominated production systems, at the expense of women's productive activities. The respondents informed as all amount of finance including the safety net money given for fulfill their food gap is less accessed by rural women.

If all access to productive resources is summed up men have 79% of access to total productive resource where as women have only 21% of access (Figure 4). This indicates as rural women have weak or less access to different productive resources. Unless different concerned bodies' reveres this access the chance of rural women to break the poverty vicious circle is less.

Implication of the result to poverty reduction

Having less access to productive resources rural women's effort towards poverty reduction is very less. All development efforts still on-going are just like clamping with one hand. The gender based division of labor leaves aside the half of societies (women's) labor. They are still playing reproductive role. The productive role and almost all of the means of production are controlled by men.

Poverty can be thought of as an "inadequate" livelihood outcome. It may be the result of the household having inadequate access to assets, like land, water, credit or social support. It can be also caused by policies, institutions and processes that are not supportive of achieving an equal access of productive resources of

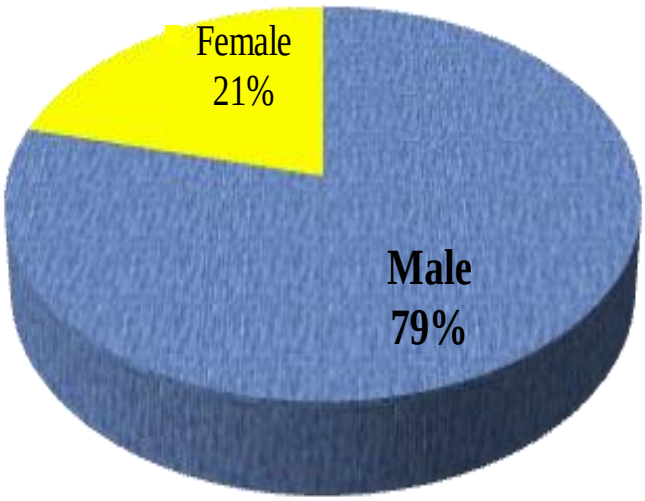

Figure 4. General access to productive resources.

both men and women. Therefore poverty can be defined simply as the combination of uncertain or non-existent income and a lack of access to productive resources needed to ensure sustainable living conditions.

In rural areas, where services and job opportunities are even fewer than in urban areas and where rural women's have very less access to productive resources, poverty is also more acute. The situation is worse for women, who are less likely to have access to production factors, services and resources such as credit, land, water for irrigation, inheritance, education, information, extension services, technology and farm inputs, as well as a say in decision-making.

Another reason for the persistence of female poverty is gender vulnerability within the home. When poor families cannot afford to send all of their children to school, they favor investing in the boy-children, keeping the girls at home to help with domestic work. In this research area as well as in many societies, inequalities of women and men were part and parcel of an accepted male-dominated culture.

Improving gender division of labor, ensuring women's access to productive resources requires an integrated approach to growth and development, focused on gender bases. Economic growth and poverty reduction strategies should give attention to the real economy and focus on creating a gender-sensitive macroeconomic environment, access to land, property and other productive resources as well as financial services, and full coverage of social protection measures.

Throughout the research area, gender inequality in access to productive resources, such as land, water, credit, technology and other means of production, is closely related to women's poverty and economic and social exclusion. While challenges to the effective enjoyment of rural women's economic rights are complex and often context-specific, there are also many shared obstacles, including their ineffective implementation of legal rights at all levels, as well as discriminatory attitudes and practices. In order to ensure that women enjoy their rights in practice, a broad conceptualization of rights and access to productive resources is needed that is sustainable, gender-responsive and inclusive of both urban and rural areas. This approach should be consistent with national human rights standards and the human rights-based approach to development.

Conclusion

Rural women are key agents for development. They play a catalytic role towards achievement of transformational economic, environmental and social changes required for sustainable development and poverty reduction. But man biased division of labor, less access to different productive resources are among the many challenges they face. Gender differences, arising from the socially constructed relationship between men and women, affect the division of labor the role they play in the community the distribution of resources between them and the benefits from different development outcomes. This is explicitly found in the agriculture sector, where gender inequalities in access to resources are persistent, undermining a sustainable and inclusive development of the sector and their contribution for poverty reduction.

RECOMMENDATIONS

An effective implementation of Ethiopia's gender empowerment policy will significantly help to improve the conditions of rural female farmers.

Without the participation of the rural poor female farmers in rural development implementation and the establishment of efficient rural organizations to act as countervailing forces to vested interests, it is unlikely that significant progress will be made in increasing access by rural women farmers to productive resources. Economic growth strategies should give attention to the real economy and focus on creating a gender sensitive microeconomic environment, full employment and decent work, access to land, property and other productive resources as well as financial services, and full coverage of social protection measures. Extension services are pivotal to increased productivity, agricultural development and poverty redaction. Therefore by any means rural women should have get chance to contact with DAs. Continuous contact with DA's can help rural farmers to get new idea and practice about the agricultural work.

Conflict of Interests

The authors have not declared any conflict of interests.

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Effect of tillage method on *Fusarium* blight severity and yield of soybean in Omu-Aran, Southern Guinea Savannah of Nigeria

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***Fusarium* blight of soybean, caused by *Fusarium oxysporum* is one of the most destructive diseases of the legume. The pathogen is difficult to control owing to its persistence in the soil and wide host range. Soil tillage practice is one of the most important components of cultural soil management techniques that have a great influence on intensity of plant diseases. This study investigated the effect of tillage practice on the severity of *Fusarium* blight and yield of soybean. The land used for the trial was artificially inoculated with inoculum suspension of *F. oxysporum* left for one week before tillage. The experiment was laid out in a randomized complete block design (RCBD) with four treatments, each replicated three times. The treatments consist of ploughing only (P), ploughing followed by harrowing (PH), harrowing only (H) and no tillage (control). The parameters assessed include disease severity, number of pods per plant, pod length, pod weight, 100 seed weight and total seed yield. Findings from this study showed that at 4 weeks after planting, the highest disease severity (1.9) was recorded in soybean planted on ploughed land while the least blight severity (0.8) was recorded with no tillage. Soybean sown under no tillage produced significantly (P<0.05) higher number of pods per plant (41.6) while the lowest pod number (23.3) were produced on soybean sown on ploughed land. Soybean sown under no tillage produced a significantly (P<0.05) higher seed yield (328.0 kg/ha) than all other treatments. Tillage practice is an effective way of managing soybean diseases owing to its potential to adjust soil temperature and moisture. The tillage methods used in the current study incorporated *Fusarium* blight pathogen at varying soil depths, with no tillage being the most effective approach of reducing the severity of soybean *Fusarium* blight in infected soil.**

Key words: Blight, *Fusarium oxysporum*, soybean, tillage, disease severity, yield.

INTRODUCTION

Soybean, *Glycine max* L. (Merr) is a leguminous crop widely cultivated in tropical, subtropical, and temperate climates of the world (IITA, 2009). The legume provides cheap and high quality protein comparable to meat,

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poultry and eggs. Soybean is the richest source of complete plant protein, containing all amino acids essential for human nutrition.

More than 216 million tons of soybeans were produced worldwide in 2007 with Africa accounting for only 1.5 million tons of this production estimate (Hartman et al., 2011). Nigeria is the largest producer and consumer of soybean in Sub-Saharan Africa with a low yield of less than 1 ton per hectare (IITA, 2009). Several abiotic and biotic constraints threaten soybean production, resulting in reduced yield and quality. The major biotic factors are weeds, pests and diseases (Hartman et al., 2011). More than 300 diseases have been reported on soybean (Hartman et al., 1999). Losses attributed to soybean diseases alone are estimated at 11% (Hartman et al., 1999).

The increase in the number of soybean diseases and their expansion emanate from intensive production and increased acreage in new regions of the world (Hartman et al., 2011). In areas where soybean is grown every year or even every other year, propagules of various types of pathogens have increased to densities that cause economic yield losses.

Fusarium blight or wilt disease of soybean, caused by the common soil-borne fungus, *Fusarium oxysporum*, is one of the most destructive diseases of soybean (Hashem et al., 2009; Fayzalla et al., 2009). The pathogen can affect soybeans at any stage of development (Ferrant and Carroll, 1981). Disease symptoms are first noticed on the lower (older) leaves. The middle and lower leaves turn yellow or have pale yellow spots. As the disease progresses, the younger leaves become affected. The upper leaves of infected plants wilt and appear scorched. Affected plants also show a wilting of the stem tips. In severe cases, the leaves dry up and drop prematurely, leaving the petiole behind (Nelson et al., 1997). *Fusarium* blight symptoms are more noticeable under reduced moisture and hot conditions. The pathogen is difficult to control owing to its persistence in the soil and wide host range (Abdel-Monaim et al., 2011).

Soil tillage practice is one of the most important components of cultural soil management techniques that have a great influence on intensity of plant diseases. The tillage practice embarked upon during land preparation could have influence on the intensity and frequency of soil-borne pathogens. Depending on soil tillage method, varying amount of plant residues remain in or on the soil surface (Jug et al., 2011) which, through interactions with other agro-ecological components has various effects on diseases (Jordan and Hutcheon, 2003).

F. oxysporum is a soil-borne fungus with infected plant debris serving as a source of inoculum for the pathogen (Arias, 2012). Although, extensive research studies have been carried out on soybean production and improvement in Nigeria, there is a paucity of information on cultural soil management techniques that influence disease intensity, severity and yield losses in soybean arising from blight (Hartman et al., 1999). The present study therefore investigated the effect of tillage practice on the severity of *Fusarium* blight and yield of soybean.

MATERIALS AND METHODS

Study site

The experiment was carried out in the Teaching and Research Farm of Landmark University, Omu-Aran, Kwara State. Omu-Aran is located in the north central part of Nigeria in the south-eastern direction of Ilorin. The site lies between latitude 8.9°N and longitude 50°61 E of the equator. The annual rainfall pattern of the area is 600 to 1,500 mm between the months of April and October, with peaks in June. The humidity ranges from 50% in the dry season to about 85% during the wet season.

Isolation of *F. oxysporum*

Roots and basal stems of naturally infected soybean plants showing wilt disease symptoms were collected from an infected field and taken to the Crop Science Laboratory of Landmark University, Omu-Aran where pathogen was isolated using procedures described by Ferrant and Carroll (1981). The samples were thoroughly washed in tap water, cut into small pieces of 0.5 cm and surface sterilized for 2 min in 2% sodium hypochlorite solution, then rewashed several times in sterilized distilled water and dried between a number of folds of sterilized filter papers. The surface sterilized samples were plated on Potato Dextrose Agar (PDA) medium supplemented with penicillin (20 µl ml^{-1}) and incubated at 25±1°C for 6 days. The developed fungal colonies were purified by single spore techniques to obtain pure culture of *F. oxysporum*.

Preparation of fungal inoculum

Disks taken from one week old culture of *F. oxysporum* prepared were inoculated on 75 ml Potato Dextrose (PD) broth medium in 250 ml flask and incubated at 25 ± 1°C. The obtained fungal suspension was collected on No. 1 Whatman filter paper and rinsed with sterile distilled water, then placed in a warring blender with a small amount of sterile water and blended for 2 min at high speed. Sterile distilled water was then added to each inoculum suspension to give a final volume of 200 ml.

Experimental design and treatment application

The land used for the trial was artificially inoculated with 100 ml of inoculum suspension of *F. oxysporum* every 50 cm apart and left for one week before tillage. The experiment was laid out in a randomized complete block design (RCBD) with four treatments, each replicated three times. The treatments consist of ploughing only (P), ploughing followed by harrowing (PH), harrowing only (H) and no tillage or control (C). Each plot measured 3.0 x 3.5 m and plots were separated from one another by 1.0 m alleys.

Soybean planting and maintenance

Three seeds of cultivar TGx 1448-2E soybean was sown per hill at

Table 1. Effect of tillage method on severity of *Fusarium* blight of soybean.

Tillage method	Disease severity				
	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
P	1.9[a]	2.1[a]	2.7[a]	3.3[a]	4.3[a]
PH	1.4[b]	1.6[a]	2.2[a]	2.6[b]	3.6[b]
H	1.3[b]	1.4[a]	2.0[a]	2.4[b]	3.5[b]
NT	0.8[c]	0.9[b]	1.2[b]	1.8[c]	2.4[c]

P=Ploughing only, PH= Ploughing + harrowing, H= Harrowing only, NT= No tillage, WAP= Weeks after planting. Values are means of three replicates. *Means in columns followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

a depth of 2 to 3 cm and at a spacing of 70 cm between the rows and 40 cm within the row in each treatment. The seedlings were thinned to one plant per stand one week after emergence. An artificial water supply and weeding were carried out regularly throughout the duration of the study.

Data collection and analysis

Six plants were randomly selected from each plot and tagged for assessment of disease severity. Commencing from four weeks after planting, scoring for disease severity was carried out at two weeks interval over a period of 10 weeks. Soybean blight severity was determined according to Abdou et al. (2001) using rating scale of 0 to 5,

0 = no yellow leaf
1 = 1-25% yellow colouration on one leaf
2 = 26-50% yellow colouration on more than one leaf
3 = 51-75% yellow colouration plus one wilted leaf
4 = 76-100% yellow colouration with more than one wilted leaf, and
5 = completely dead plants.

Data were collected from each plot on the following yield components; number of pods per plant, pod length, pod weight, 100 seed weight. At harvest, seeds obtained from the two inner rows in each plot were weighed separately to obtain total seed yield. The data collected was subjected to analysis of variance (ANOVA) and the means were separated using Duncan's Multiple Range Test (DMRT) at 5% probability level.

RESULTS

Disease severity

Tillage method had a significant effect on severity of *Fusarium* blight of soybean (Table 1). At four weeks after planting, the highest disease severity (1.9) was recorded in soybean planted on ploughed land while the least blight severity (0.8) was recorded on the treatment with no tillage. There was no significant difference (P≤0.05) in *Fusarium* blight severity in soybean plants in the ploughed + harrowed and harrowed lands. At six and eight weeks after sowing, no significant differences in disease severity were noticed in soybean plants grown in ploughed, ploughed + harrowed and harrowed lands.

At ten and twelve weeks after sowing, the highest disease severities were recorded on plants in the ploughed land and this was significantly higher than all other treatments. No significant difference in *Fusarium* blight severity was observed in soybean plants in the ploughed + harrowed and harrowed lands.

Table 2. Effect of *Fusarium* blight of soybean on number of pods per plant.

Tillage method	Number of pods per plant
P	23.3[c]
PH	34.6[b]
H	35.7[b]
NT	41.6[a]

P=Ploughing only, PH= Ploughing + harrowing, H= Harrowing only, NT= No tillage, WAP= Weeks after planting. Values are means of three replicates. *Means in columns followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

Number of pods produced per plant

Soybean sown under no tillage produced a significantly (P≤0.05) higher number of pods per plant (41.6) while the lowest pod number (23.3) were produced on soybean sown on ploughed land (Table 2).

Pod length

Effect of *Fusarium* blight of soybean on pod length is presented in Table 3. The result shows that soybean sown under no tillage had a significantly higher pod length (16.0 cm) than all other treatments. The pod length was not significantly (P≤0.05) influenced by *Fusarium* blight on soybean plants sown on ploughed, ploughed + harrowed and harrowed lands.

Pod weight

Results of the effect of *Fusarium* blight of soybean on

Table 3. Effect of *Fusarium* blight on soybean pod length (cm).

Tillage method	Pod length (cm)
P	12.8[b]
PH	13.4[b]
H	13.6[b]
NT	16.0[a]

P=Ploughing only, PH= Ploughing + harrowing, H= Harrowing only, NT= No tillage, WAP= Weeks after planting. Values are means of three replicates.*Means in columns followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

Table 4. Effect of *Fusarium* blight on pod weight (kg).

Tillage method	Pod weight (kg)
P	1.7[a]
PH	1.8[a]
H	1.7[a]
NT	2.1[a]

P=Ploughing only, PH= Ploughing + harrowing, H= Harrowing only, NT= No tillage, WAP= Weeks after planting. Values are means of three replicates.*Means in columns followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

Table 5. Effect of *Fusarium* blight on 100 seed weight (g).

Tillage method	100 seed weight(g)
P	10.1[a]
PH	10.3[a]
H	10.3[a]
NT	10.5[a]

P=Ploughing only, PH= Ploughing + harrowing, H= Harrowing only, NT= No tillage, WAP= Weeks after planting. Values are means of three replicates.*Means in columns followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

Table 6. Effect of *Fusarium* blight on soybean seed yield per hectare (kg/ha).

Tillage method	Seed yield(kg/ha)
P	216.7[c]
PH	244.2[b]
H	254.8[b]
NT	328.0[a]

P=Ploughing only, PH= Ploughing + harrowing, H= Harrowing only, NT= No tillage, WAP= Weeks after planting. Values are means of three replicates.*Means in columns followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

pod weight is presented in Table 4. *Fusarium* blight did not significantly influence the weight of pods in all the treatments, although numerically higher pod weight (2.1 kg) was recorded with no tillage.

100 seed weight

Results on the effect of *Fusarium* blight of soybean on 100 seed weight is presented in Table 5. The result shows that *Fusarium* blight did not significantly influence 100 seed weight in all the treatments.

Total seed yield

Effect of *Fusarium* blight of soybean on seed yield per hectare is presented in Table 6. Soybean sown under no tillage produced a significantly (P≤0.05) higher seed yield (328.0 kg/ha) than all other treatments. The least yield of 216.7 kg/ha was recorded in soybean plants on ploughed land. There was no significant difference in the yields of soybean in ploughed+harrowed land and harrowed land only, although numerically higher seed yield per hectare (254.8 kg) was obtained in the latter.

DISCUSSION

The present study investigated the effect of tillage method on severity of *F. oxysporum* and the concomitant effect on yield components and yield of soybean. Findings from this trial revealed that the tillage method embarked upon during land preparation influenced disease severity, number of pods produced per plant, pod length and total yield.

Fusarium species are widespread soil-borne organisms capable of surviving for long periods of time as chlamydospores and as mycelium in plant residues and in the soil (Arias, 2012). Infected plant debris serve as a source of inoculum for the pathogen. Cool temperatures and wet soils, particularly early in the growing season, often favour infection by *Fusarium* species (Marasas et al., 1984; Nelson et al., 1997; Yang, 1997). As soil moisture becomes more limiting, soybeans may become stressed, thereby increasing susceptibility to infection by *Fusarium* (Zhang et al., 2010). The reduced moisture condition of the soil during the growing period in this study could have triggered the susceptibility of the crop to *Fusarium* blight.

Tillage practices have varying efficacies on disease management. Soil tillage practices involving various depths, intensity, and different methods of loosening the soil and treating plant residues can significantly influence plant diseases (Vanova et al., 2011). The residues may be an infection source for several important diseases caused by fungi of the *Fusarium* genus (Váňová et al., 2009a). The pathogens have a chance to stay in the soil,

reproduce and spread. Depending on tillage method, different amount of plant residues remain on the soil surface (Jug et al., 2011) which, through interactions with other agro-ecological components, has various effects on pests, diseases and weeds (Jordan and Hutcheon, 2003).

Since many plant pathogens can survive on plant debris, ploughing has traditionally been used to incorporate crop residues (Poštić et al., 2012). *Fusarium* blight pathogen was incorporated at varying soil depths during the tillage operation. Soybean plants on ploughed land had the highest disease severity (Table 1). The lowest disease severity observed with no tillage in this study agrees with the findings of Perez-Brandan et al., (2012) that reported good soil quality, increased level of microbial activity, nutrient cycling, microbial diversity and enhanced natural disease suppression under zero tillage. Krupinski et al., (2002) also reported that no till reduces many crop diseases because of their direct and beneficial effects on soil biology. A healthy soil with diverse and balanced populations of soil micro-organisms will provide substantial competition against root pathogens as these often use the same organic carbon substrate. Furthermore, Yang (2008) reported that soybean cyst nematode and some soil-borne diseases, such as *Rhizoctonia* root rot, would not be reduced by tillage practices. Indeed, tillage practices increase the movement of soybean cyst nematode and further spread the risk of soil-borne pathogens.

Several soybean diseases prevalent in some areas can be effectively controlled with tillage practices while some cannot (Yang, 2008). Tillage methods have proved very effective in reducing the risk of many foliar and stem diseases of soybean, such as *Cercospora* leaf spot, brown spot, frog-eye leaf spot, downy mildew, bacterial blight, brown stem rot, and *Phomopsis*. Pathogens of these diseases survive in crop residues in the absence of soybean crop. When infested crop residues are buried in soil, their decomposition rate increases and the fungi die.

As such, this tillage approach reduces the amount of pathogens that survive to the next crop season.

Soil moisture is usually higher and temperatures are cooler in conservation tillage systems than in conventional tillage systems. These factors, especially cooler temperatures, could have caused a reduction in disease development with no tillage as observed in this trial. This is corroborated by the findings of Porter and Wright (1991) who reported that warmer temperatures favour the development of *Cercospora arachidicola*, causal organism of early leaf spot of peanut. The researchers observed that pod yields were greater in conventional tilled plots even though leaf spot was less in conservational tilled plots.

Conclusion

Tillage practice is an effective way of managing several crop diseases owing to its potential to adjust soil temperature and moisture. The tillage methods used in the current study incorporated *Fusarium* blight pathogen at varying soil depths, with no tillage being the most effective approach of reducing the severity of soybean *Fusarium* blight in infected soil. Increased number of pods and yield were also obtained with no tillage.

Conflict of Interests

The authors have not declared any conflict of interests.

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Nutrients dynamics in soil solution at the outset of no-till implementation with the use of plant cocktails in Brazilian semi-arid

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Tillage systems strongly impact nutrient transformations and plant availability. Therefore, the objective of this study was to assess the impacts of conversion of conventional tillage (CT) to no-till (NT) with a mixture of cover crops and green manure as nutrient uptake in a fertilized melon (*Cucumis melon*) in a semi-arid region of Brazil. Two fields experimental involved randomized blocks design, in a split-plot scheme, with four replication treatments included three types of cover crops and two tillage systems (conventional and no-till). Subsamples of plant cocktails were used to assess the biomass production. Soil samples were analyzed during the melon growth for determination of soil moisture by the frequency domain reflectometry (FDR) probe. Soil solution samples were extracted with ceramic cups from each treatment, and analyzed for determination of TP, Na^{+}, Ca^{2+}, Mg^{2+}, S and NO_3-N. Mobility of these elements was assessed in relation to management and different cover crops. The data showed slight or no strong effect of plant cocktails composition on nutrients dynamics in soil under melon. However, without incorporation of biomass and slower decomposition of residue mulch retained on the surface, risks of leaching losses were lower under NT than CT system. A higher concentration of cations in CT (for example, Ca^{+2} ~ 42.07 mg L^{-1}) may be attributed to high soil moisture content and faster rate of mineralization of the biomass incorporated. Concentration of P was higher in top soil layers depth in NT system (~ 6.65 mg L^{-1} at 15 cm) because of the deposition of plant cocktail biomass in soil surface with low SOM contents placement of fertilizer, and possible formation of calcium phosphate with low solubility. Relatively, high concentration of NO_3-N (~ 60.16 mg L^{-1}) in CT was attributed to increase in decomposition of soil organic matter (SOM) and crop residues incorporated into the soil.

Key words: Macronutrient, soil fertility, cover crop, soil management, *Cocumis melo, Caatinga*.

INTRODUCTION

Soils of the semi-arid regions have been prone to degradation because of change in land cover associated with different land uses, mismanagement, and harsh climate (Lal, 2004). In the semi-arid regions of Brazil,

conversion of the natural thorn forest (caatinga) into arable land is causing loss of soil organic matter (SOM), depletion of nutrients, and accelerated erosion (Wick et al., 2000). Thus, sustainability of land use systems depends on adoption of conservation agriculture (CA) methods which use cover crops to generate enough dry biomass to provide a continuous soil cover throughout the year. Thus, a mixture of cover crops, known as plant cocktail, has been evaluated for uses as cover crops and green manure in semi-arid regions of Brazil (Giongo et al., 2011).

The use of plant cocktails as cover crops can recycle nutrients from the sub-soil the surface (Carvalho et al., 2011). In addition, residues of plants cover conserves soil water by reducing runoff and evaporation, increasing water storage in the effective rooting depth, increasing plant-available water capacity, and increasing net primary production by reducing risks of drought and decreasing losses of plant nutrients by runoff, leaching and erosion (Lal, 2013).

Bohnen and Da Silva (2006) observed that no-till (NT) system changed the dynamics of nutrients in the soil in relation to conventional tillage, especially over a long-term period, although alterations in the system were observed soon after the conversion, with important effects on nutrient availability to plants. Information about composition of the soil solution may be useful in relation to environmental management, soil fertility dynamics, and plant growth (Zambrosi et al., 2008). Bohnen and Da Silva (2006) observed that higher concentrations of Ca^{2+}, Mg^{2+} PO_4^-, and K^+ were observed in surface soil layers even during the first year of conversion to NT. Ionic concentrations are affected by soil type and tillage system, and formulation of nitrogen fertilizers influence the water flux and the concentration of NO_3-N in soil solution (Sangoi et al., 2003). The reduction of water evaporation under cover crop residues in no-till systems also accentuates the downward movement of nitrate via macropores (Muzilli, 1983). Yet, high NO_3-N leaching is also observed in conventional till system, but it is attributed to the greater decomposition of SOM and of the crop residues incorporated in the soil than that in the NT system (Bayer and Mielniczuck, 1997). High concentrations of NO_3-N were also observed in the fertigated treatments, and indicated large potential for N loss by leaching (Souza et al., 2012).

Among several factors affecting nutrient movement in soil are: concentration in soil solution, adsorption capacity of the soil (Qafoku et al., 2000), loads of the complex ion exchange (Qafoku and Sumner, 2001), pH (Qafoku et al., 2000), solubility of fertilizer (Shuman, 2001), soil water content (Padilla et al., 1999) and the soil macroporosity (Shipitalo et al., 2000).

The objective of this research was to evaluate the beginning of conversion to NT with reference to the conventional tillage, and determine the effect of plant cocktails used as cover crops and green manure, in a fertilized melon (*Cucumis melo* L.) growth under semi-arid conditions of Brazil.

METHODOLOGY

The field experiment on melon was conducted at the Bebedouro Experimental Farm (latitude 09009'S, longitude 40022'W and altitude 365.5 m), Embrapa Semi-Arid (Brazilian Agricultural Research Corporation) from October to December, 2012. Before this experiment, the site was used for research on date palm crop (*Phoenix dactylifera*). There was no application of liming. The soil is classified as Ultisol dystrophic red-yellow plinthic (EMBRAPA, 2011). It has a high sand concentration of 74.87% of 0.0 to 0.2 m depth, with a gentle trend of decrease in sand content to 0.8 m depth. Thus, different soil layers are classified as sandy loam for 0.4 to 0.6 and sandy clay loam for 0.8 to 1.0 m depth (Silva et al., 2001). Analysis of composite soil samples were obtained from the experimental site for 0.0 to 0.2 m depth, according to the standard methods recommended by EMBRAPA (2011), before initiating the experiment and showed the following physical and chemical mean: CEC 0.57 ± 0.17 cmolc dm^{-3}; pH (H_2O) of 6.1 ± 0.2; P (Mehlich 1) of 46.12 ± 2.11 mg dm^{-3}; H+Al 2.14 cmolc dm^{-3}; the exchangeable value of K^+, Na^+, Ca^{2+} and Mg^{2+} of 0.36 ± 0.01, 0.03 ± 0.01, 2.33 ± 0.15, 0.43 ± 0.16 cmolc dm^{-3}, respectively; the sum of bases (S) of 3.16 ± 0.16 cmolc dm^{-3}, and base saturation (V) of 59.6 ± 1.53% (Table 1).

The climate is classified as Bswh according to the Köppen classification system, with an average annual temperature of 26.8°C, an average annual rainfall of 360 mm, and the climax vegetation called Caatinga (xeric shrubland and thorn forest). Data of air temperature (maximum and minimum), evapotranspiration and precipitation were measured at the agrometeorological weather station located at Bebedouro Experimental Farm. Plant cocktails were established in the beginning of July before the growing of melon. Melons were planted at row spacing of 0.5 m. By the end of September plant cocktails effective as a cover crop were maintained and the other parts were incorporated by a disc harrow to 40 cm depth. The treatments were arranged in four blocks in a split-plot design. Two tillage treatments as main plots had dimensions of 30 × 20 m. Conventional tillage (CT) comprised of plowing and disking compared with no soil disturbance in NT plots. Sub-plots treatments, 10 × 10 m, comprised three cropping systems (two different compositions of Plant cocktail and one natural vegetation cover): NTC1 - 75% legumes + 25% non-legumes and NT; NTC2 - 25% of legumes + 75% non-legumes and NT; NTNV - natural vegetation and NT; TC1 - 75% legumes + 25% non-legumes and CT; TC2 - 25% legumes + 75% non-legumes and CT; TNV - natural vegetation and CT. Plant species already used as green manure and cover crops adapted to semi-arid were used in this experiment. Fourteen species included in the composition of Plant cocktails, comprised legumes, oilseeds and grasses, including the

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Table 1. Results of soil analysis of composite samplings from the Bebedouro Experimental Field. Standard deviation values in brackets.

	E.C.	Ph (H_2O)	P	K	Na	Ca	Mg	Al	H+Al	S (Base)	CEC	V
Depth (m)	**$dS.m^{-1}$**		**$mg.dm^{-3}$**					**$cmolc/dm^3$**				%
0-20	0.57	6.10	46.12	0.36	0.03	2.33	0.43	0.50	2.14	3.16	5.30	59.67
	(0.17)	(0.20)	(2.11)	(0.01)	(0.01)	(0.15)	(0.06)	(0.0)	(0.0)	(0.16)	(0.16)	(1.53)

following species: A) Legumes - calopo (*Calopogonium mucunoide*), velvet bean (*Stizolobium aterrimum* L.), grey-seeded mucuna (*Stizolobium cinereum* Piper e Tracy), crotalaria (*Crotalaria juncea*), rattlebox (*Crotalaria spectabilis*), jack beans (*Canavalia ensiformes*), pigeon pea (*Cajanus cajan* L.), lab-lab bean (*Dolichos lablab* L.); B) no legumes: sesame (*Sesamum indicum* L.), corn (*Zea mays*), pearl millet (*Penissetum americanum* L.) and milo (*Sorghum vulgare* Pers.) sunflower (*Helianthus annuus*), castor oil plant (*Ricinus communis* L.). The natural vegetation was composed by the predominant species: benghal dayflower (*Commelina benghalensis* L.), purple bush-bean (Macroptilium atropurpureum), florida beggarweed (*Desmodium tortuosum*) and goat's head (*Acanthorpermun hispidum* DC).

Subsamples of plant cocktails from each treatment were weighted and sent to the Laboratory of Soil (Embrapa semiarid), stored in a greenhouse at 65 to 70°C for 72 h, and weight again (g kg^{-1}) was recorded to estimate the dry matter yield (Mg ha^{-1}).

Melon seeds were planted in a substrate under greenhouse and seedlings were transplanted in the field about 10 to 12 days after emergence of the first permanent leaves. One seedling per hole was transplanted at spacing of 0.3 × 2.0 m. Drip irrigation was used for both plant cocktail and melon crop. In plant cocktail, plastic pipes were distributed between the rows with drip emitters spaced at 0.5 m which provided a low flow rate of 4.0 L h^{-1}. In melon, the same plastic pipes and drip emitters were distributed between the rows with 2.0 m width. Thus, the amount of water applied was the same for all treatments and was determined on the basis of the evapotranspiration (ETo) as determined by the Class A pan evaporation (ECA). During the 70 days growth period of melon, all treatments were equally fertilized according to the specific recommendations at the rate of 38.0 kg CO(NH2)2 ha-1 (Urea - 45% N) applied 16 times, 16.0 kg KCl ha-1(60% K) applied 15 times, 67.0 kg Ca(NO3)2 ha-1 (15%N and 19%Ca) applied 5 times, 100.0 kg P2O5 ha-1 applied 8 times and 20.0 kg (NH4)H2PO4 ha-1(MAP) applied 15 times.

Dynamics of macronutrients in soil solution was studied by obtaining samples of soil solution in middle and at the end of the melon growth cycle. A PVC (1.27 cm) extractor with ceramic caps at the upper end and a fixed silicone tube for suction of soil solution were used as lysimeter. The soil solution was extracted 24 h after irrigation. This lysimetric installation consisted of 24 batteries of 3 extraction units of the soil solution. These units were installed one for each treatment in the experimental field blocks in the row at 0.15, 0.30, and 0.50 m depth. Ceramic cups were washed and immersed in deionized water until the time of installation in the field. Soil solution samples were collected in plastic bottles, properly labeled and stored at 4°C pending analyses. Soil solution samples were analyzed for total phosphorus (TP), Na^+, Ca^{2+}, Mg^{2+} and S by inductively coupled plasma optical emission spectrometry technique (ICP-OES, Perkin Elmer, USA) and NO_3-N by flow injection analysis method (FIA).

While soil solution sample were obtained at 3 times during the growing season of melon, nutrients concentration in the bulk samples were measured only for a composite sample because of the short growing cycle of only 65 to 70 days. Soil moisture content was measured to 40 cm depth at three times during the melon season: beginning of October, middle of November and middle of December, 2012. A segmented FDR probe (PR2 model - Delta T Devices) with a Dataloger HH2 moisture meter was used by installing 24 sets of 2 access tubes (1.0 m long) on the crop rows for each treatment. Soil moisture measurements were made to 0.4 m depth, which is the effective rooting depth of melon. In seasonal melons, growth in the northeast of Brazil have an effective rooting depth of 30 cm (Mota et al., 2008).

All the results were statistically analyzed for variance (ANOVA), using the ASSISTAT – free statistical program (version 7.7 beta - Federal University of Campinas Grande-Brazil). The difference between treatment means was assessed by the Tukey test, at 5 % probability.

RESULTS AND DISCUSSION

Meteorological data

The amount of precipitation received during the experimental period was small, and occurred only at the beginning of November. A high precipitation of 6.86 mm was received on November 2nd. The mean temperature during the growth period of sampling was about 28°C with the maximum of 31.06°C recorded on December 4th and minimum of 25.32°C recorded on October 1st. The pan evapotranspiration ranged from 3.71 to 8.15 mm during the growing period (Figure 1). Because of low precipitation, high temperature, and evapo-transpiration, the melon crop was irrigated every 2 days. Thus, precipitation had no influence on nutrients dynamics in soil for any treatments. Therefore, only irrigation and fertigation processes were considered as the main factors, followed by temperature and cover crop. Photodegradation is an important determinant of above-ground litter decomposition in this semi-arid ecosystem (Austin and Vivanco, 2006). The high temperature increases evatranspiration, soil metabolism process and organic matter mineralization. Thus, the principal concern is the leaching of nitrogen (Stuart et al., 2011).

Biomass yield

Figure 2 shows the dry matter (DM) for the 2 types of cocktail plant and natural vegetation. The average DM yield was 9.71 (±1.97), 10.24 (±2.85) and 5.71 (±2.51) Mg ha^{-1} for plant cocktail 1, plant cocktail 2 and natural

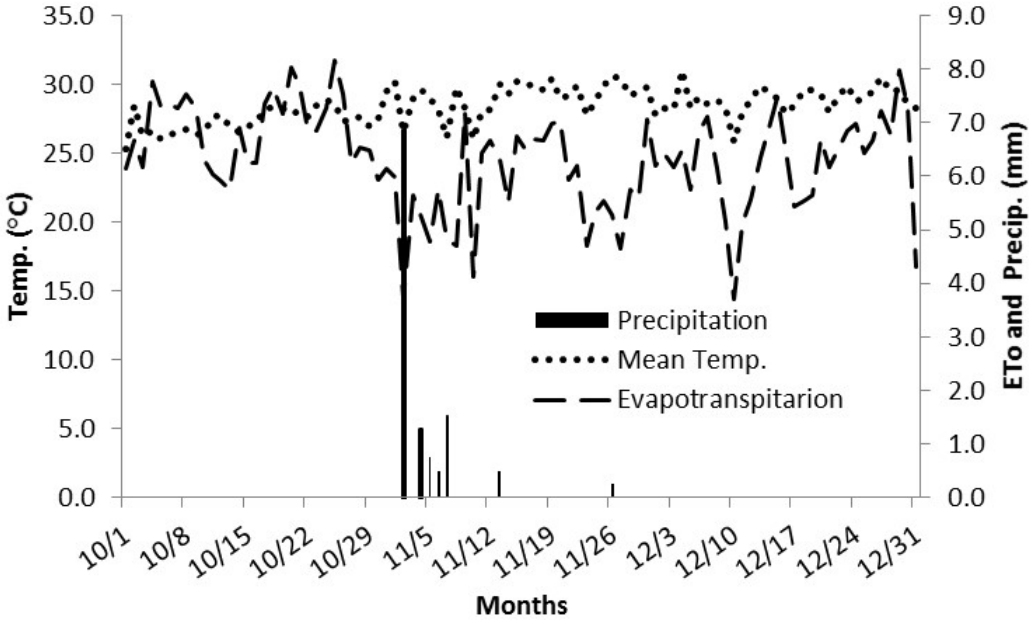

Figure 1. Mean temperature, reference evapotranspiration and precipitation in Bebedouro Experimental Field – Embrapa Semi-arid, during the period of October to December, 2012.

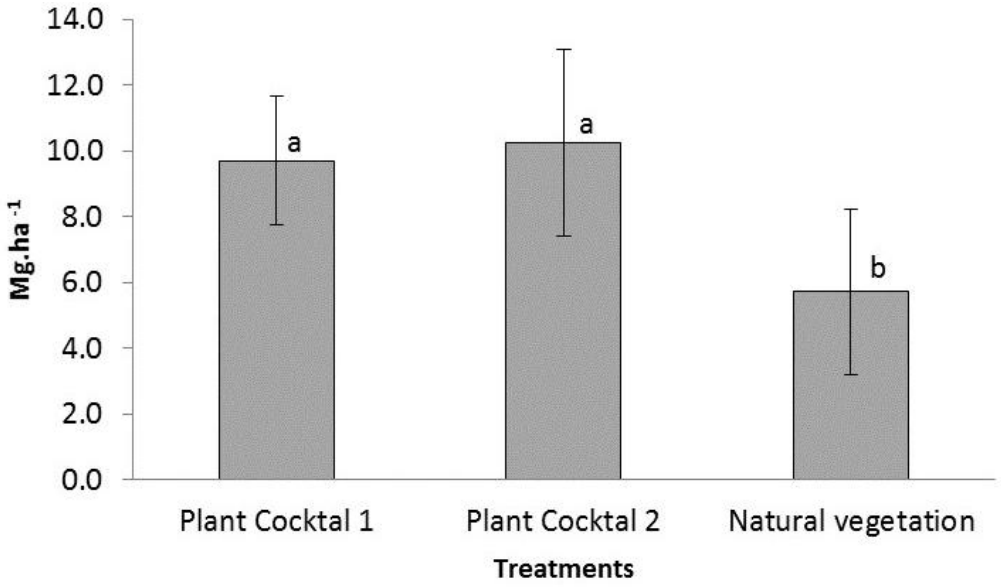

Figure 2. Dry matter yield from plant cocktails 1 and 2 compared with natural vegetation. Error bars show the standard deviation of the means. Means followed by the same letter are not significantly different by Tukey test at $P < 0.01$. LSD = 3.11 and CV% = 28.8.

vegetation, respectively. These results show the efficacy of these species as cover crops for semi-arid conditions. About 6.0 Mg ha^{-1} of plant residues is needed to provide an effective soil cover under a NT system (Alvarenga et al., 2001). However, the optimum amount may differ among plant species and edaphoclimatic conditions. The biomass produced by plants cocktails influences soil conditions, reduces nutrient losses by leaching and

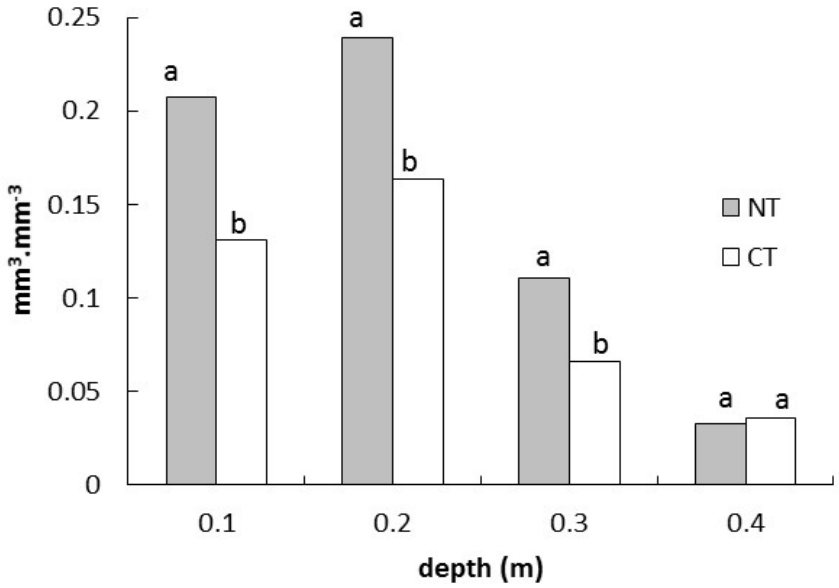

Figure 3. Variation of moisture in soil profile considering the mean of treatments under no-till (NT) and conventional tillage (CT) with the use of cocktail plants in Brazilian semi-arid. Means followed by the same letter are not significantly different by Tukey test at $P < 0.05$. LSD = 0.043

erosion, maintaining soil moisture, increases water infiltration, and reduces weed growth, recycles nutrients, especially when legume species are used, and improves soil structure especially when grasses are used (Carvalho et al., 2010). The time required to decompose half of the dry biomass of plant cocktails ranged from 116 to 173 days, depending on soil management. Relatively higher decomposition rate was observed in all plant cocktails managed with the CT (data not presented).

Soil moisture content

The soil moisture content in 0.2 m depth was higher in all treatments under NT than that CT conventional tillage, principally to depth of 0.20 m of the profile. Overall to 30 cm depth, soil moisture contents under NT treatments were significantly different than those under CT (Figure 3). In general, soils under NT store more water in the surface layer (Panachuki et al., 2015). The higher water retention in NT is attributed to the maintenance of cover crop on soil surface, which acts as a barrier, reducing water loss by evaporation (Ward et al., 2013). Despite obtaining three soil solution samples during the melon crop, only an average nutrients concentration of different layers were considered because of the short life cycle of around 65 to 70 days. Therefore, nutrients mobility and accumulation in the soil layers were verified with relation to soil management changes with different types of cover crops under drip fertigation.

Soil solution concentration

Despite of no liming, the treatments with CT (mainly TC1 and TC2) had higher concentration of Ca^{+2} in 15 cm depth (47.50 to 48.71 mg L^{-1}) than that in NTC1, NTC2 and NTNV, because of low pH, adoption of NT and low mineralization under NT than CT. Taking average concentration for two management systems (M-NT and M-CT), Ca^{+2} concentrations was 42.21 ± 34.51 mg L^{-1} under CT (Figure 4 and Table 2), and there were no significant differences among treatments for 30 cm soil depth, but trends of values were observed in the soil profile (52.97 to 65.07 mg L^{-1}).

Use of Ca $(NO_3)_2$ as fertilizer can produce a stable NO_3-N anion upon solubilization, increasing leaching of Ca^{+2} as an accompanying ion, and maintains chemical neutrality of the salt front through mass flow in soil (Ziglio and Miyazawa, 1999). Mass flow is the primary mechanism of supplying Ca^{+2}, thus soil soil solution concentration is a major factor governing this process (Silva et al., 2006). Higher soil-water content within the 30 cm layer can leach out Ca^{+2} increase in its concentration in sub-soil layers. However, mixing under of plant biomass in CT accentuates the rate of mineralization under NT system and affects the release of water-soluble organic anions, altering pH and

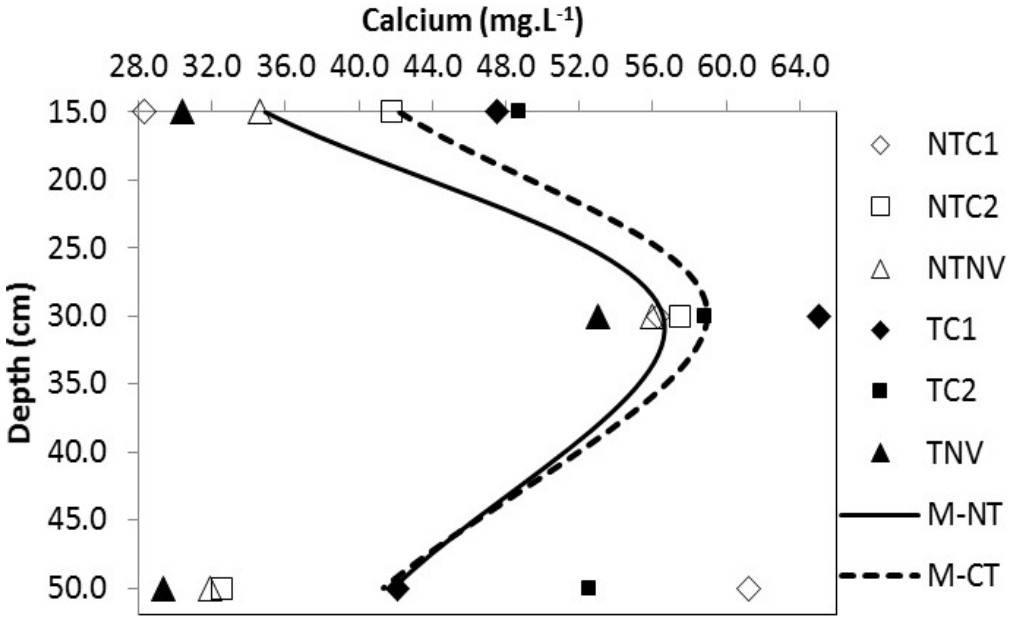

Figure 4. Concentration of calcium in the soil solution at depths of 15.0. 30.0 and 50.0 cm from two cropping systems and three different cover crop. NTC1. no-till and plant Cocktail 1; NTC2. no-till and plant Cocktail 2; NTNV. no-till and Natural vegetation; TC1. Conventional tillage and cocktail 1; TC2. Conventional tillage and cocktail 2; TNV. Conventional tillage and Natural vegetation; M-NT. means of no-tillage treatments; M-CT. means of conventional tillage treatments.

Table 2. Calcium concentration in soil solution at depths of 15.0. 30.0 and 50.0 cm for all the treatments.

Calcium (mg L^{-1})	Depth (cm)		
	15.0	**30.0**	**50.0**
NTC1	28.29^{bB}	56.24^{aA}	61.24^{aA}
NTC2	41.75^{abB}	57.49^{aA}	32.56^{cB}
NTNV	34.64^{abB}	55.92^{aA}	31.88^{cB}
TC1	47.50^{aB}	65.07^{aA}	42.07^{bcB}
TC2	48.71^{aA}	58.81^{aA}	52.54^{abA}
TNV	30.40^{bB}	52.97^{aA}	29.33^{cB}
M-NT	34.90 (29.38)	56.55 (34.59)	41.90 (18.85)
M-CT	42.21 (34.51)	58.95 (35.66)	41.32 (18.22)

Values followed by the same letter do not differ by Tukey test at 5% probability. Columns - lower case (LSD = 15.80); Lines - capital letters (LSD = 12.96). CV% = 23.01.

enhancing the mobilization of Ca^{+2} within the soil (Silva et al., 2006). The highest concentrations of Ca^{+2} observed at 50 cm soil depth (42.07 mg L^{-1}) was under NRC1, but these mean concentrations of Ca^{+2} at this depth were similar among all treatments. There were significant differences in Ca^{+2} concentrations at 15 and 50 cm depths of TC1 and TC2 than that of TNV, probably because of the mineralization of plant cocktails biomass incorporated into the soil, which is higher than that under the native vegetation regrowth.

Both Ca^{+2} and Mg^{+2} cations have a similar behavior in soil (Stinner et al., 1984). Thus, a proportional concentration of those cations was computed. The data show that moderate amounts of Mg^{+2} were leached from the top soil to 50 cm depth (Figure 5). However, no significant differences were observed among treatments and depth. Similar to Ca^{+2}, concentrations of Mg^{+2} was also the lowest at 15 cm depth, and mean concentration ranged from 3.77 mg L^{-1} in TNV to 6.67 mg L^{-1} in NTNV. Concentrations of Mg^{+2} were high at 30 cm depth in all

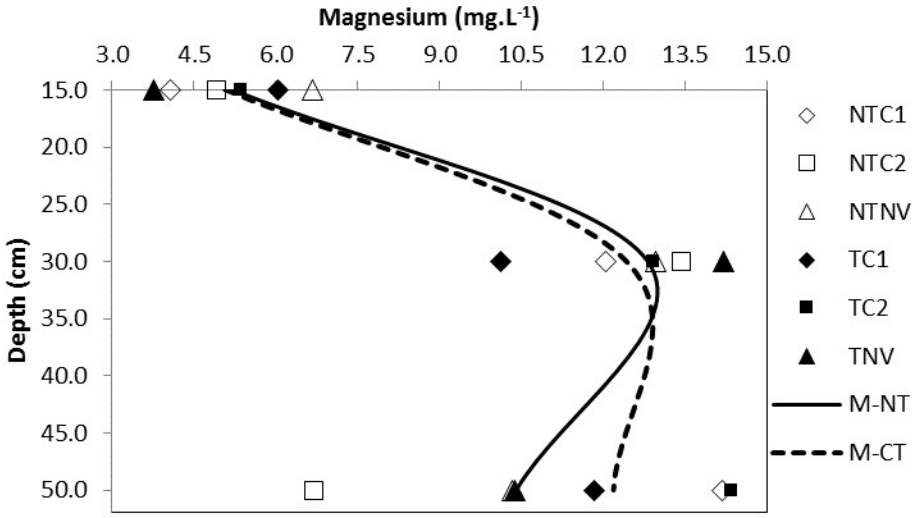

Figure 5. Concentration of magnesium in the soil solution at depths of 15.0. 30.0 and 50.0 cm from two cropping systems and three different cover crop. NTC1. no-till and plant Cocktail 1; NTC2. no-till and plant Cocktail 2; NTNV. no-till and Natural vegetation; TC1. Conventional tillage and cocktail 1; TC2. Conventional tillage and cocktail 2; TNV. Conventional tillage and Natural vegetation; M-NT. means of no-tillage treatments; M-CT. means of conventional tillage treatments

Table 3. Magnesium concentration in soil solution at depths of 15.0. 30.0 and 50.0 cm for all the treatments.

Magnesium (mg L^{-1})	Depth (cm)		
	15.0	**30.0**	**50.0**
NTC1	4.07^{aB}	12.03^{aA}	14.17^{aA}
NTC2	4.91^{aB}	13.43^{aA}	6.69^{bB}
NTNV	6.67^{aB}	12.95^{aA}	10.33^{abAB}
TC1	6.03^{aB}	10.12^{aA}	11.82^{aA}
TC2	5.35^{aB}	12.91^{aA}	14.33^{aA}
TNV	3.77^{aC}	14.19^{aA}	10.38^{abB}
M-NT	5.22 (5.69)	12.81 (9.15)	10.40 (5.16)
M-CT	5.06 (7.20)	12.41 (8.21)	12.18 (5.91)

Values followed by the same letter do not differ by Tukey test at 5% probability. Columns - lower case (LSD = 4.63); Lines - capital letters (LSD = 3.81). CV% = 40.68.

treatments, and the highest concentration of 14.19 mg L^{-1} in TNV. These trends indicate high mobility of Mg^{+2} in the soil followed by that of Ca^{+2} (Table 3).

The mean concentration of Na^{+} in soil solution reached from 3.81 to 8.16 mg L^{-1}, and there were no significant differences among treatments for 15 and 30 cm depths. Mean concentration of Na^{+} for treatments in the same management system (M-NT; M-CT) indicated similar values for different soil depths. However, concentration of Na^{+} in soil solution was slightly higher for TC1 and TC2 than that for NT treatments (NTC1 and NTC2), and the mean concentration ranged from 3.95 to 5.61 mg L^{-1} (Figure 6, Table 4). Tillage and crop residue management can strongly affect water relations and leaching of soluble salt (Dalal, 1989). Similar concentrations of Na+ were observed in all treatments probably because of a soil moisture content in all depths. The highest of concentration of > 8.0 mg L^{-1} was recorded at 50 cm depth. Salt accumulation in the profile is primarily controlled by the amount of salts released and leached from the soil and the amount of salts leaving the soil by percolation (Gupta and Abrol, 1990).

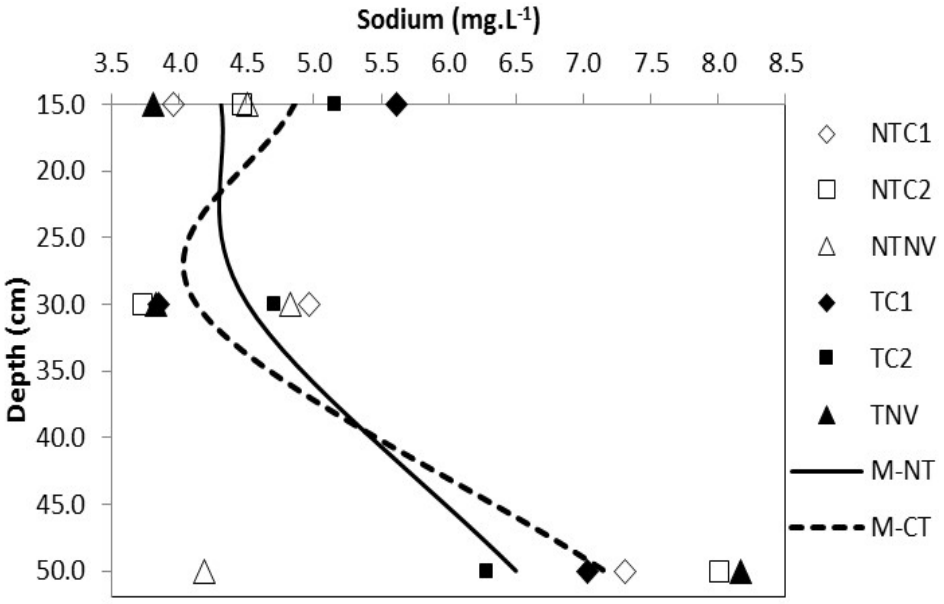

Figure 6. Concentration of sodium in the soil solution at depths of 15.0. 30.0 and 50.0 cm from two cropping systems and three different cover crop. NTC1. no-till and plant Cocktail 1; NTC2. no-till and plant Cocktail 2; NTNV. no-till and Natural vegetation; TC1. Conventional tillage and cocktail 1; TC2. Conventional tillage and cocktail 2; TNV. Conventional tillage and Natural vegetation; M-NT. means of no-tillage treatments; M-CT. means of conventional tillage treatments.

Table 4. Sodium concentration in soil solution at depths of 15.0. 30.0 and 50.0 cm for all the treatments.

Sodium (mg L^{-1})	Depth (cm)		
	15.0	**30.0**	**50.0**
NTC1	3.95^{abB}	4.96^{aB}	7.30^{abA}
NTC2	4.46^{abB}	3.72^{aB}	8.00^{abA}
NTNV	4.51^{abA}	4.82^{aA}	4.18^{cA}
TC1	5.61^{aA}	3.85^{aB}	7.02^{abA}
TC2	5.15^{abAB}	4.70^{aB}	6.27^{bA}
TNV	3.81^{bB}	3.83^{aB}	8.16^{aA}
M-NT	4.31 (2.21)	4.51 (2.34)	6.50 (2.88)
M-CT	4.86 (2.56)	4.13 (1.99)	7.16 (3.81)

Values followed by the same letter do not differ by Tukey test at 5% probability. Columns - lower case (LSD = 1.75); Lines - capital letters (LSD = 1.44); CV% = 28.35.

There were no significant differences among treatments in SO_4^{-2} concentration for 15 and 50 cm depth, and the mean concentration ranged from 10.34 ± 4.52 (M-CT) to 10.99 ± 4.34 (M-NT). In general, in SO_4^{-2} on agrosystem is rapidly cycled and easily leached (Silva et al., 1999). Despite the highest SO_4^{-2} concentration observed at 50 cm depth in the present study, high concentration of 14.27 mg L^{-1} (TEV), at 15 cm depth indicates its low mobility (Figure 7 and Table 5). Because at low mobility of SO_4^{-2} compared with Cl, N etc, it moves in soil by mass flow in the water (Vitti et al., 1994). When sulfur is not added in the soil, any slight increase in soil solution is attributed to mineralization of biomass and SOM (Miranda et al., 2006) and its leaching along with water. Despite lack of any significant differences among treatments, the CT treatments trended to have higher SO_4^{-2} concentration below 30 cm depth, because of decomposition of incorporated biomass and high soil moisture content. Stratification in SO_4^{-2} may also occur during early stages than in long-term condition of NT (Crozier et al., 1999).

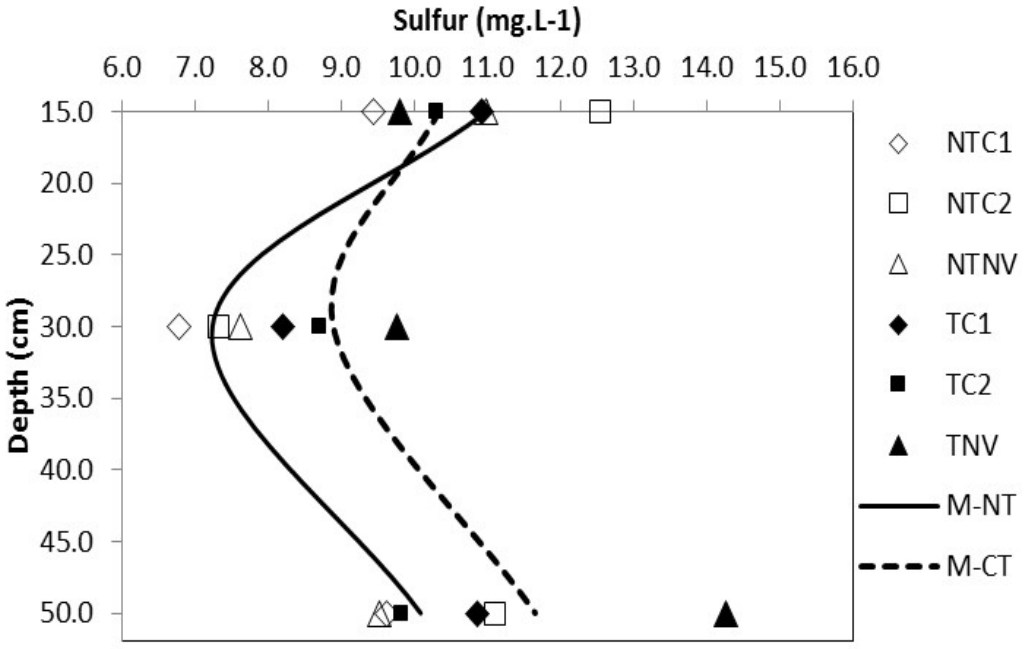

Figure 7. Concentration of sulfur in the soil solution at depths of 15.0. 30.0 and 50.0 cm from two cropping systems and three different cover crop. NTC1. no-till and plant Cocktail 1; NTC2. no-till and plant Cocktail 2; NTNV. no-till and Natural vegetation; TC1. Conventional tillage and cocktail 1; TC2. Conventional tillage and cocktail 2; TNV. Conventional tillage and Natural vegetation; M-NT. means of no-tillage treatments; M-CT. means of conventional tillage treatments.

Table 5. Sulfur concentration in soil solution at depths of 15.0. 30.0 and 50.0 cm for all the treatments.

Sulfur (mg L^{-1})	Depth (cm)		
	15.0	**30.0**	**50.0**
NTC1	9.43^{bA}	6.78^{bB}	9.61^{bA}
NTC2	12.54^{aA}	7.31^{abB}	11.11^{bA}
NTNV	10.98^{abA}	7.61^{abB}	9.53^{bAB}
TC1	10.91^{abA}	8.20^{abB}	10.86^{bA}
TC2	10.29^{abA}	8.69^{abA}	9.82^{bA}
TNV	9.79^{bB}	9.75^{aB}	14.27^{aA}
M-NT	10.99 (4.34)	7.24 (3.12)	10.09 (5.07)
M-CT	10.34 (4.52)	8.89 (2.29)	11.65 (3.75)

Values followed by the same letter do not differ by Tukey test at 5% probability. Columns - lower case (LSD = 2.67); Lines - capital letters (LSD = 2.19); CV% = 23.01.

Expectedly, the P concentrations varied strongly with soil depth from 6.65 mg L^{-1} at 15 cm to 0.13 mg L^{-1} (NTC2) at 50 cm soil depth (Figure 8). The highest P concentrations recorded in topsoil indicated its low mobility in soil profile. There were significant differences in P concentrations among NT treatments (NTC1, NTC2 and NTEV) and CT treatments (TC1, TC2 and TEV) (Table 6). Despite high value of P concentration in the surface layer, there were no significant differences between NT and CT at 30 cm depth. Because of minimal soil erosion in NT and the location of fertilizer, high accumulation of P in the surface layer can be 10 times compared to that in the surface layers (Muzilli, 1983; Rheinheimer et al., 1998).

Soil of the experimental site is slightly acidic, and thus has a low potential of formation of SOM in treatments other than NT. Under these conditions of soil pH approaching to neutral value, soluble phosphorus is

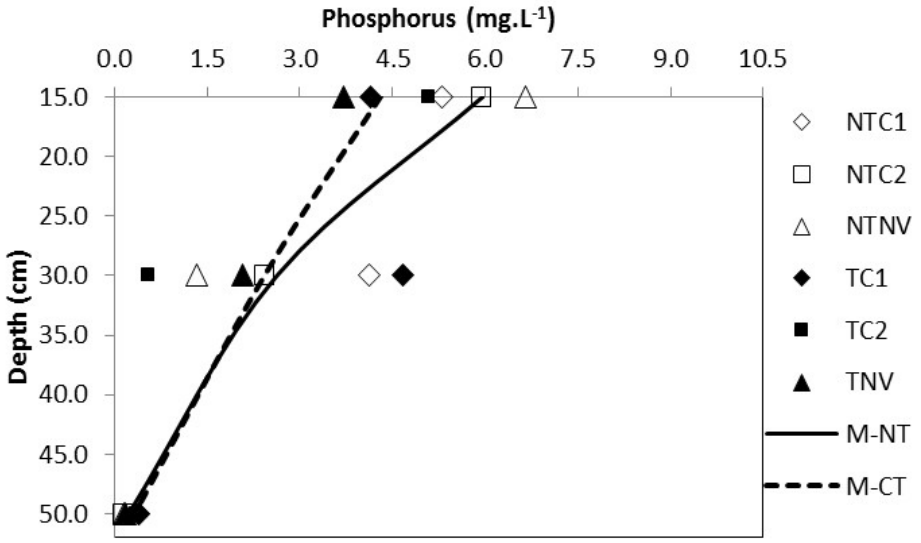

Figure 8. Concentration of phosphorus in the soil solution at depths of 15.0. 30.0 and 50.0 cm from two cropping systems and three different cover crop. NTC1. no-till and plant Cocktail 1; NTC2. no-till and plant Cocktail 2; NTNV. no-till and Natural vegetation; TC1. Conventional tillage and cocktail 1; TC2. Conventional tillage and cocktail 2; TNV. Conventional tillage and Natural vegetation; M-NT. means of no-tillage treatments; M-CT. means of conventional tillage treatments.

Table 6. Phosphorus concentration in soil solution at depths of 15.0. 30.0 and 50.0 cm for all the treatments.

Phosphorus (mg L^{-1})	Depth (cm)		
	15.0	**30.0**	**50.0**
NTC1	5.31^{bcA}	4.12^{aB}	0.40^{aC}
NTC2	5.93^{abA}	2.43^{bB}	0.13^{aC}
NTNV	6.65^{aA}	1.33^{bcB}	0.16^{aC}
TC1	4.16^{cdA}	4.67^{aA}	0.4^{aB}
TC2	5.08^{bcA}	0.56^{cB}	0.35^{aB}
TNV	3.72^{dA}	2.07^{bB}	0.19^{aC}
M-NT	5.96 (4.34)	2.63 (2.10)	0.23 (0.23)
M-CT	4.32 (2.13)	2.43 (2.14)	0.31 (0.25)

Values followed by the same letter do not differ by Tukey test at 5% probability. Columns - lower case (LSD = 1.29); Lines - capital letters (LSD = 1.06); CV% = 41.50.

transformed into low solubility form of calcium phosphate in the soil surface (Souza et al., 2012). Nonetheless, drip fertilization can increase PO_4^{-3} movement into the sub-soil compared to that with the conventional application because of concentration of the soil in a narrow range, which quickly saturates soil in vicinity of the zone of application (Villas Boas et al., 1999). However, that process depends of soil attributes and the specific formulation used (Souza et al., 2012).

Mean concentration of NO_3-N ranged from 19.45 mg L^{-1} at 15 cm to 60.16 mg L^{-1} at 50 cm soil depth, indicating high leachability (Figure 9). However, no significant differences were observed between NT and CT treatments for 15 cm depth, albeit a high value of 42.14 mg L^{-1} was recorded for TC2. The high soil moisture content at ~ 30 cm depth concentrated high NO_3-N in this layer in all treatments, with average value of 54.27 (43.10) mg L^{-1} to NT and 54.62 (43.97) mg L^{-1} to CT. At 50 cm depth, however, higher NO_3-N concentration is observed in TC1 (60.16 mg L^{-1}) and TC2 (59.19 mg L^{-1}) treatments (Table 7). Bayer and Mielniczuck (1997) observed more leaching of NO_3-N in CT system because

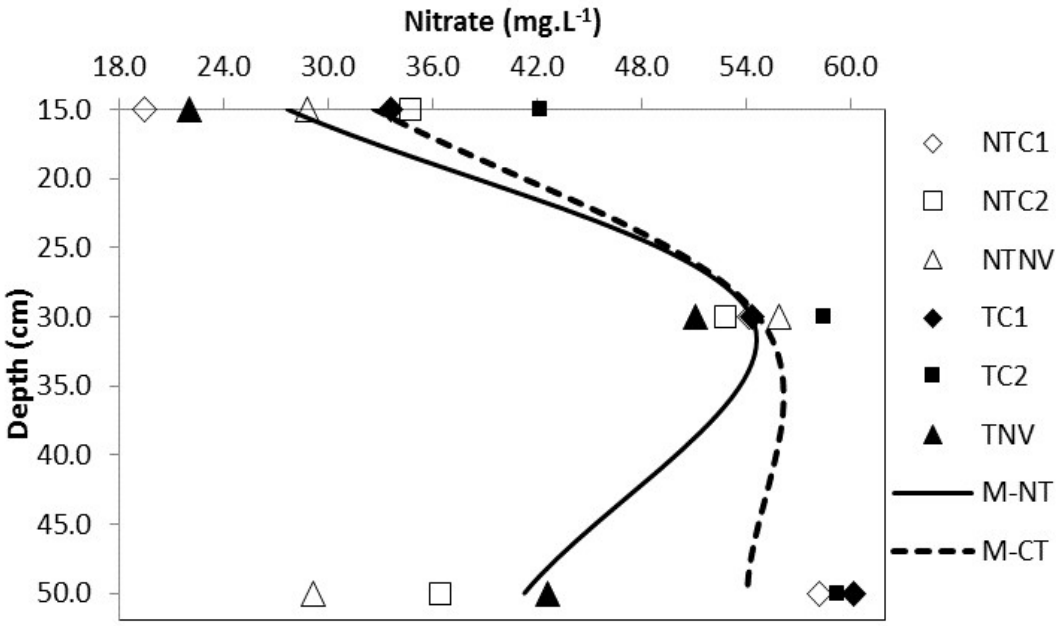

Figure 9. Concentration of nitrate in the soil solution at depths of 15.0. 30.0 and 50.0 cm from two cropping systems and three different cover crop. NTC1. no-till and plant Cocktail 1; NTC2. no-till and plant Cocktail 2; NTNV. no-till and Natural vegetation; TC1. Conventional tillage and cocktail 1; TC2. Conventional tillage and cocktail 2; TNV. Conventional tillage and Natural vegetation; M-NT. means of no-tillage treatments; M-CT. means of conventional tillage treatments.

Table 7. Nitrate concentration in soil solution at depths of 15.0. 30.0 and 50.0 cm for all the treatments.

Nitrate ($mg.L^{-1}$)	Depth (cm)		
	15.0	**30.0**	**50.0**
NTC1	19.45^{bB}	54.16^{aA}	58.21^{abA}
NTC2	34.66^{abB}	52.82^{aA}	36.37^{cB}
NTEV	28.76^{abB}	55.83^{aA}	29.13^{cB}
TC1	33.57^{abB}	54.29^{aA}	60.16^{aA}
TC2	42.14^{aB}	58.48^{aA}	59.19^{aA}
TEV	22.03^{bB}	51.10^{aA}	42.61^{bcA}
M-NT	27.62 (28.49)	54.27 (43.10)	41.24 (22.06)
M-CT	32.58 (32.58)	54.62 (43.97)	53.99 (26.69)

Values followed by the same letter do not differ by Tukey test at 5% probability. Columns - lower case (LSD = 16.08); Lines - capital letters (LSD = 13.19). CV% = 30.99.

of increased decomposition of SOM and crop residues incorporated in the soil compared to the NT system. Leaching of NO_3-N below the rooting depth of melon is a major concern. Therefore, a split application of fertilizer can reduce leaching losses in sand soils.

Stinner et al. (1984) observed that concentrations of NO_3-N were the highest in CT those in NT soils. Indeed, nitrification is reduced in NT compared with that CT soil because NH_4-N is the predominant form of N in NT soil (Souza et al., 2012). In addition, use of $Ca(NO_3)_2$ with drip fertigation leads to a uniform distribution of NO_3-N in the soil profile (Haynes, 1990). Leaching of NO_3-N requires presence of accompanying cations, while the protons produced by ammonium nitrification or organic by nitrogen are remain in the surface layer as a source of potential acidity (Franchini et al., 2000). The data from this study indicate between the cations ($Ca^{+2}$and Mg^{+2}) and the anion (NO_3-N) for all the treatments and soil

Table 8. Correlation between the concentrations of cations (calcium and magnesium) and nitrate for all the treatments.

Treatment	Equation[a]	r^2
NTC1	Cations = 0.9611 N-NO_3^-+ 13.621	0.77*
NTC2	Cations= 0.9568 N-NO_3^-+ 13.854	0.79*
NTNV	Cations = 0.9554 N-NO_3^-+ 13.98	0.81*
TC1	Cations = 0.9528 N-NO_3^-+ 14.117	0.82*
TC2	Cations = 0.9611 N-NO_3^-+ 13.308	0.84*
TNV	Cations = 0.9539 N-NO_3^-+ 13.825	0.82*
Total[b] Cations = 0.9611 N-NO_3^- + 13.308 0.84*		

[a]Considering the three depths. [b]Considering the 6 treatments in three depths. *Significant t test P < 0.001.

depths studied (r^2 = 0.84; p < 0.001) (Table 8), suggesting that Ca^{+2} and Mg^{+2} are the accompanying cations. The use of $Ca(NO_3)_2$ as fertilizer produces $Ca^{+2}$and Mg^{+2} which accentuates the mobility of Ca^{+2} and Mg^{+2} and maintains chemical neutrality of the salt front by mass flow (Ziglio and Miyazawa, 1999).

Conclusions

The data presented support the following conclusions:

(i) There was either slight or no strong effect of plant cocktails composition on nutrients dynamics in soil under melon. Perhaps, the short time of melon growing cycle crop was not long enough to cause a substantial mineralization of the cocktail biomass. Nonetheless, some changes were observed with the adoption of NT system.
(ii) Without incorporation of biomass and slower decomposition of residue mulch retained on the surface, risks of leaching losses were lower under NT then CT system.
(iii) The higher concentrations of cations (that is, Ca^{+2}) in CT may be attributed to a high soil moisture content and faster rate of mineralization of the biomass incorporated.
(iv) In general, S had a low mobility. Concentration of S was high in CT from 30 cm depth because of the high rate of decomposition of plants biomass incorporated and high soil moisture content.
(v) Concentration of P was higher in top soil layers depth in NT system, because of the deposition of plant cocktail biomass in soil surface with low SOM contents placement of fertilizer, and possible formation of calcium phosphate with low solubility.
(vi) Concentration of NO_3-N was high and large amount were leached into the sub-soil. However, high concentration of NO_3-N in CT may be attributed to increase in decomposition of SOM and crop residues incorporated into the soil.

Conflict of Interests

The authors have not declared any conflict of interest

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Full Length Research Paper

Seedling and adult plant resistance to leaf rust in some Egyptian wheat genotypes

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Leaf rust of wheat caused by *Puccinia triticina* Eriks. is one of the most widespread disease in Egypt. In this study, thirteen Egyptian wheat genotypes were evaluated for leaf rust resistance at seedling stage under greenhouse condition and adult plant stage under field conditions over three growing seasons that are, 2011/2012, 2012/2013 and 2013/2014 and three locations that are, Itay El-Baroud and Nubariya Agricultural Research Stations as well as the Farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom. The tested wheat genotypes were classified into three groups according to their resistance. The first group, race-specific resistant genotypes including Shandweel 1, Misr 1, Misr 2, Sids 12 and Sids 13, showed the lowest values of final rust severity (FRS %) and area under disease progress curve (AUDPC). The second group, slow-rusting or partially resistant genotypes including Sakha 94, Gemmeiza 9, Giza 168, Sakha 95, Gemmeiza 10 and Gemmeiza 11, displayed low level of FRS and AUDPC. The third one which includes Gemmeiza 7 and Sids 1, showed the highest values of FRS and AUDPC. Postulation of leaf rust resistance genes was differed between the tested genotypes.Results indicated that Sakha 95 and Sids 12 may have seven resistance genes. Moreover, Gemmeiza 10 may has five genes and Misr 1 may has three genes. While, Giza 168, Sids 1, Misr 2 and Shandweel 1 may have two genes. The wheat genotypes Gemmeiza 11 and Gemmeiza 12 may have only single gene. Also, all the tested wheat genotypes may contain some additional genes.In contrast, the wheat genotypes Sakha 94, Gemmeiza 7, Gemmeiza 9 and Sids 13 did not have any of the tested genes.

Key words: Wheat, leaf rust, seedling resistance, adult plant resistance, final rust severity (FRS), area under disease progress curve (AUDPC), gene postulation.

INTRODUCTION

Leaf rust caused by *Puccinia triticina* Eriks. is a widespread disease of wheat (*Triticum aestivum* L.) in Egypt and worldwide. Yield losses due to leaf rust disease may be more than 50% for some susceptible wheat genotypes (German et al., 2007). Breeding wheat genotypes with resistance to leaf rust is the most effective control method and environment friendly approach to reduce the yield losses (Winzeler et al., 2000).

At present, more than 80 genes and alleles of leaf rust resistance genes have been identified and described. Among them 33 *Lr* genes were transferred from other

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species into *Triticum aestivum* L. (Herrera-Foessel et al., 2011, 2012; Ingala et al., 2012; McIntosh et al., 2012). Most of the resistance genes are effective at seedling stage and remain effective through the adult plant stage. Some of the leaf rust resistance genes express resistance optimally in adult plants and are known as adult plant resistance (APR) genes, which depends on the genetics of the host pathogen interaction as well as favored environmental conditions.

Rust resistance in wheat has been based on the use of race specific resistance genes. But the short-lived nature of race specific hypersensitive resistance has created the necessity to search for the more durable type of resistance. Several researchers reported that some genotypes showed the ability to retard the rust development even though they had a susceptible reaction type (Caldwell et al., 1970; Singh et al., 1991).This type of resistance known as slow rusting resistance.

Avoiding major rust epidemics in the region is a complex challenge, given that fewer genotypes are being cultivated over large areas, and several of those genotypes are protected by the same resistance genes. To identify those genotypes with resistant sources that are the most fit for the cultivation in the more diseased areas of the country, genotype screening for leaf rust resistance is considered the best and the cheapest method.

The objectives of this research work are to determine the resistance of some Egyptian wheat genotypes at seedling and adult plant stages to leaf rust through postulating and identifying resistance genes in the tested wheat genotypes.

MATERIALS AND METHODS

Seedling studies

Genotypes evaluation and resistance genes postulation of the tested wheat monogenic lines were carried out at seedling stage in the greenhouse of Wheat Diseases Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt.

A total of 107 leaf rust samples were collected during three successive growing seasons. Forty samples were collected in 2010/2011, thirty eight samples in 2011/2012 and twenty nine samples in 2012/2013 (Table 1) from the wheat commercial fields and the trap nurseries grown in different locations of Egypt. These locations were Beheira (31 samples), Dakahlia (12), Gharbiya (10), Minufiya (12), Sharqiya (20), Domiata (3), Qalyubia (5) and Bani Swif (14). Sample (2 to 4 infected leaves) was kept at room temperature (18 to 24°C) overnight to be dried off then kept in glycine envelopes (8 × 15 cm) and stored with deiscator in the refrigerator at 2 to 5°C. The infected specimens were transferred through inoculation to the highly susceptible variety; Thatcher for isolation and purification.

The method of inoculation was carried out as described by Stakman et al. (1962), in which wheat seedling leaves (7 days old) were rubbed gently between moisted fingers with tap water, sprayed with water in the incubation chambers.Then inoculated by shaking or brushing rusted materials from collected samples over the plant leaves and sprayed gently again with water in order to form initial film of free water on the plants which is essential for spore germination and establishment of infection. The inoculated seedlings were incubated in humid chambers for 24 h to allow spore germination and cause infection. The inoculated plants were moved onto the benches in the greenhouse with daily temperature 10 to 25°C. After approximately 12 to 15 days, three single pustules were isolated separately from each sample for rust reproduction on seedlings of the highly susceptible wheat variety Thacher to obtain enough urediniospores for inoculation.

Race designations were assigned as described by Long and Kolmer (1989) including 16 differential lines, each with a single leaf rust resistance gene from one of four subsets, first (*Lr* 1, *Lr* 2a, *Lr* 2c and *Lr* 3a), second (*Lr* 9, *Lr* 16, *Lr* 24 and *Lr* 26), third (*Lr* 3ka, *Lr* 11, *Lr* 17 and *Lr* 30), fourth (*Lr* 10, *Lr* 18, *Lr* 21 and *Lr* 2b). Supplemental near-isogenic lines containing *Lr* 14b, *Lr* 15, *Lr* 36 and *Lr* 42 were also inoculated at the same time as additional differential sub-set from Egypt suggested by McVey et al. (2004).

At the same time, seeds of the tested wheat genotypes i.e. Sakha 94, Sakha 95, Giza 168, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Gemmeiza 11, Sids 1, Sids 12, Sids 13, Misr 1, Misr 2 and Shandweel 1 were sown in 6 cm square plastic pots. Five seeds of each tested wheat material were sown in each corner in a clockwise order. Seven days old seedlings of the tested wheat materials, when first leaf full emerged, were inoculated with single pustule isolates of *P. triticina* which was previously propagated. Inoculation was carried out by shaking with propagated urediniospores over the seedling leaves of the tested materials. The inoculated seedlings were transferred onto the greenhouse benches(18 to 20°C and 100% RH).

Seedling disease assessment

Infection type (IT) data for each tested wheat genotype were recorded 12 days after inoculation using standard infection type scoring scale 0 to 4 (Stakman et al., 1962). Genotypes which showed low infection types (scores = 0, 0; 1, and 2) were considered as resistant or low infection types (LITs). While, those with scores = 3 and 4 were susceptible or high infection types (HITs) (Stakman et al., 1962).

Virulence frequency

Virulence frequency was calculated as percentage of virulent isolates to the total number of the tested isolates.

$$\text{Virulence frequency (\%)} = \frac{\text{No. of virulent isolates}}{\text{Total number of isolates}} \text{ X } 100$$

$$\text{Level of varietal resistance (\%)} = \frac{\text{No. of avirulent isolates}}{\text{Total number of isolates}} \text{ X } 100$$

Postulation of leaf rust resistance genes

Thirteen Egyptian wheat genotypesand 39 monogenic lines carrying single gene for leaf rust resistance were tested in seedling stage using 15pathotypes of leaf rust i.e. BBCHS, BCCMS, BJTJG, CJNBD, JFKTS, LCJKB, LMSDB, MHCQQ, NSHLQ, PBBFB, PTQTT, QHJHJ, RJCRQ, SCLKB and SKPSS. All plant materials were grown in plastic pots.Seedlings were inoculated by urediniospores of the selcted identified pathotypes during 2013/2014 growing season. The inoculated seedlings were incubated also as previously mentioned and transferred onto benches of the greenhouse. Both inoculation and incubation procedures were done according to the method described by Tervet

Table 1. Percentage of virulence frequency of *P. triticina* isolates and level of varietal resistance to thirteen Egyptian wheat genotypes during three successive growing seasons at seedling stage.

Genotype	2011/2012					2012/2013					2013/2014				
	No. of virulent isolates	No. of avirulent isolates	Total No. of isolates	Virulence frequency (%)*	Level of varietal resistance (%)**	No. of virulent isolates	No. of avirulent isolates	Total No. of isolates	Virulence frequency (%)	Level of varietal resistance (%)	No. of virulent isolates	No. of avirulent isolates	Total No. of isolates	Virulence frequency (%)	Level of varietal resistance (%)
Sakha 94	16	101	117	13.68	86.32	115	51	166	69.28	30.72	40	21	61	65.57	34.43
Sakha 95	61	57	118	51.69	48.31	110	55	165	66.67	33.33	25	31	56	44.64	55.36
Giza 168	75	43	118	63.56	36.44	148	18	166	89.16	10.84	47	14	61	77.05	22.95
Gemmeiza 7	84	34	118	71.19	28.81	120	46	166	72.29	27.71	45	16	61	73.77	26.23
Gemmeiza 9	91	25	116	78.45	21.55	103	63	166	62.05	37.95	48	12	60	80.00	20.00
Gemmeiza 10	102	16	118	86.44	13.56	118	48	166	71.08	28.92	44	17	61	72.13	27.87
Gemmeiza 11	40	74	114	35.09	64.91	102	64	166	61.45	38.55	48	13	61	78.69	21.31
Sids 1	74	44	118	62.71	37.29	106	60	166	63.86	36.14	38	17	55	69.09	30.91
Sids 12	13	105	118	11.02	88.98	29	137	166	17.47	82.53	14	47	61	22.95	77.05
Sids 13	7	110	117	5.98	94.02	88	78	166	53.01	46.99	44	17	61	72.13	27.87
Misr 1	2	115	117	1.71	98.29	9	157	166	5.42	94.58	8	52	60	13.33	86.67
Misr 2	10	107	117	8.55	91.45	17	149	166	10.24	89.76	35	26	61	57.38	42.62
Shandweel 1	52	65	117	44.44	55.56	71	95	166	42.77	57.23	44	17	61	72.13	27.87
No. of collected samples***				40				38					29		

*Virulence frequency (%)= Percentage of *P. triticina* isolates virulent to each wheat variety to the total number of tested isolates;** Level of varietal resistance (%): Estimated as the percentage of virulent isolates to the total number of tested isolates;***Total No. of collected samples = 107.

and Cassel (1951). Leaf rust disease infection type (IT) datawere recorded for the wheat testedmaterials as mentioned before using disease assessmentapproaches previously suggested by Stakman et al. (1962).

Field studies

Field work was carried out at three locations that are, Itay El-Baroud and Nubariya Agricultural Research Stations as well as the Farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom during 2011/2012, 2012/2013 and 2013/2014. Each of the previously mentioned thirteen wheat genotypes were planted in aplot (3 m X 3.5 m= 10.5 m^2) consisting of seven rows, and each row was 3 m long and 40 cm apart. Whole experimental plots were surrounded by spreader plants of one meter width sown with a mixture of the highly susceptible wheat genotypes to leaf rust that is, Thatcher and Morocco. The spreader plants were artificially inoculated with a mixture of urediniospores and talcum powder(1:20 v/v) of the most prevalent and aggressive fifteen leaf rust physiologic pathotypes previously mentioned. The methods of inoculation were described by Tervet and Cassel (1951).

Adult plant disease assessment

Percentage of final rust severity (FRS %)

Percentage of leaf rust severity was recorded for the thirteen wheat genotypes using the modified Cobb's scale described by Peterson et al. (1948). Rust severity data were scored after the appearance of the first symptoms (appear of the first pustule on any of the tested wheat genotypes) at seven days intervals. The percentage final rust severity (FRS %) was assessed according to Das et al. (1993), as the percentage disease severity for each tested genotypes when the highly susceptible check genotype(Sids 1) was severely rusted and the disease rate reached the highest level of leaf rust severity.

Area under disease progress curve (AUDPC)

AUDPC was also calculated for each genotype under field conditions. The values of AUDPC were calculated by using the following equation of Pandey et al. (1989).

$$AUDPC = D\,[1/2\,(Y_1 + Y_k) + (Y_2 + Y_3 + \text{- - - - -} + Y_{k-1)}]$$

Where: D = Days between two consecutive recording (time intervals)
$Y_1 + Y_k$ = Sum of the first and last scores.

$Y_2 + Y_3 + - - - - - + Y_{k-1}$ = Sum of all in between disease scores.

Statistical analysis

Least significant difference (LSD at 5 %) test was performed to determine the significant differences between means according to Steel and Torrie (1980).

RESULTS

Evaluation of the tested wheat genotype sat seedling stage under greenhouse condition

Percentage frequency of virulence to the tested wheat genotypes

Wheat genotypes Misr 1, Sids 13, Misr 2, Sids 12 and Sakha 94 showed low virulence frequencies that is, 1.71, 5.98, 8.55, 11.02 and 13.68%, respectively. While, Gemmeiza 7, Gemmeiza 9 and Gemmeiza 10 showed the highest percentage of virulence frequencies that is 71.19, 78.45 and 86.44%, respectively. In comparison, the other tested genotypes were intermediate (Table 1) in 2011/2012 growing season.

During the second growing season 2012/2013, the wheat genotypes Misr 1, Misr 2 and Sids 12 showed the lowest percentage ofvirulence frequencies that is, 5.42, 10.24 and 17.47%, respectively. While, Gemmeiza 10, Gemmeiza 7 and Giza 168 showed the highest percentage of virulence frequency that is, 71.08, 72.29 and 89.16, respectively. The other tested genotypes were extremely low to intermediate (Table 1).

In 2013/2014 growing season, both ofMisr 1 and Sids 12 wheat genotypes showed low percentage of virulence frequency i.e. 13.33 and 22.95, respectively. While, Gemmeiza 9 and Gemmeiza 12 (each with 80.00% frequency) as well as Gemmeiza 11 (78.69%) and Giza 168 (77.05) showed the highest virulence frequencies. Comparatively, the other tested wheat genotypes showed low to intermediate percentage of virulence frequencies (Table 1).

Level of varietal resistance to Puccinia triticina isolates

Data in Table 1 also revealed that five wheat genotypes Sakha 94 (86.32%), Sids 12 (88.98%), Misr 2 (91.45%), Sids 13(94.02%) and Misr 1 (98.29%) gave the highest levels of resistance (more than 80%) against the tested isolates during 2011/2012 growing season. While, Sids 12 (82.53%), Misr 2 (89.76%), Misr 1 (94.58%) and Gemmeiza 12 (100%) showed high level of resistance against 166 tested isolates of *P. triticina* collected in 2012/2013 growing season. In 2013/2014 growing season, only genotype Misr 1 gave the highest resistant reaction and exhibited 86.67%.

Postulation of leaf rust resistance genes (Lr's)

Low and high infection types displayed by the thirteen tested wheat genotypes (Table 1) compared with the infection types of 39 known *Lr* genes (Table 2) against fifteen identified tested pathotypes of *P. triticina* under greenhouse condition. Data obtained in Tables 3 and 4 were summarized in Table 3 in which different genes could be postulated as follows: The wheat genotype Sakha 95 probably possessed *Lr* 12, *Lr* 13, *Lr* 15, *Lr* 22a, *Lr* 26, *Lr* 35, *Lr*B. Wheat genotype Sids 12 may have seven genes that is, *Lr* 15, *Lr* 23, *Lr* 25, *Lr* 28, *Lr* 29, *Lr*30 and *Lr* 37. Moreover, the wheat genotype Gemmeiza 10 probably has four genes that is, *Lr* 11, *Lr* 17, *Lr*26 and *Lr* 29. The wheat genotypeMisr 1 may have three genes that is, *Lr* 27, *Lr*36 and *Lr* 37. While, the wheat genotypes Giza 168, Sids 1, Misr 2 and Shandweel 1 may have two genes. These genes were *Lr*26 and *Lr*29 in Giza 168, *Lr*23 and *Lr*29 in Sids 1 and *Lr* 2c and *Lr* 29 in Misr 2. While, wheat genotype Gemmeiza 11 may hasone gene that is, *Lr* 29. The wheat genotypes Sakha 94, Gemmeiza 7, Gemmeiza 9 and Sids 13 did not have any of the tested *Lr* genes under study using the fifteen tested pathotypes of *P. triticina* but it may carry some additional genes. Moreover, all of the tested wheat genotypes may be carries some additional gene (s).

Data in Table 4 indicated that only 19 resistance genes that are, *Lr* 2c, *Lr* 11, *Lr* 12, *Lr* 13, *Lr* 15, *Lr* 17, *Lr* 22a, *Lr* 23, *Lr* 25, *Lr* 26, *Lr* 27, *Lr* 28, *Lr* 29, *Lr* 30, *Lr*33, *Lr* 35, *Lr* 36, *Lr* 37 and *Lr* B out of the total 39 tested genes proved to be the most common genes that were probably detected and showed percentage of gene frequency from 6.66 to 46.66% of the tested wheat genotypes. Moreover, *Lr* 29 was the most frequent gene that was probably present in seven genotypes (represented 46.66% frequency), followed by *Lr*15, *Lr* 23, *Lr* 26 and *Lr* 37 (each with 20.00% frequency). While, the 20 leaf remaining rust resistance genes *Lr* 1, *Lr* 2a, *Lr* 2b, *Lr* 3, *Lr* 3ka, *Lr* 3bg, *Lr* 9, *Lr* 10, *Lr* 14a, *Lr* 14b, *Lr* 16, *Lr* 18, *Lr* 19, *Lr* 20, *Lr* 21, *Lr* 22b, *Lr* 24, *Lr* 32, *Lr* 34 and *Lr* 42 were not detected or postulated in any of the tested wheat genotypes.

Evaluation of the tested wheat genotypes under field conditions

Percentage of final rust severity (FRS %)

Data in Table 5 showed that there are significant differences in FRS means among the tested wheat genotypes. Meanwhile, there are no significant differences in means of FRS of environments as well as the interaction between genotypes and environments.

Table 2. Infection types of thirteen Egyptian wheat genotypes against fifteen pathotypes of *P. triticina* under greenhouse condition during 2013/2014 growing season at seedling stage.

Genotype	Leaf rust pathotypes / Leaf rust infection type*														
	BBCHS	BCCMS	BJTJG	CJNBD	JFKTS	LCJKB	LMSDB	MHCQQ	NSHLQ	PBBFB	PTQTT	QHJHJ	RJCRQ	SCLKB	SKPSS
Sakha 94	H***	L**	L	H	L	L	H	H	L	L	L	H	L	H	L
Sakha 95	L	H	L	L	H	L	L	L	L	L	H	H	L	L	L
Giza 168	L	H	L	L	H	H	H	L	L	L	L	H	L	H	L
Gemmeiza 7	L	H	H	L	H	H	H	L	L	H	H	H	H	H	L
Gemmeiza 9	H	H	L	H	H	H	H	L	H	L	H	H	H	H	L
Gemmeiza 10	L	L	L	L	H	H	H	L	L	L	L	H	L	L	L
Gemmeiza 11	L	H	H	L	H	H	H	L	H	H	L	H	L	L	L
Sids 1	H	H	L	L	L	L	H	L	H	L	L	H	L	L	H
Sids 12	H	H	H	L	L	L	L	L	L	L	L	L	L	L	L
Sids 13	H	H	L	L	L	L	L	L	H	H	H	H	L	L	L
Misr 1	H	L	L	H	L	L	L	L	H	L	L	L	L	L	L
Misr 2	L	L	L	L	H	L	L	L	H	H	H	L	L	L	L
Shandweel 1	H	H	L	H	H	H	L	L	H	L	L	L	L	H	L

* Infection type as described by Stakman et al. (1962);** L= Low infection type (0, 0; 1 and 2);*** H= High infection type (3 and 4).

Table 3. Infection type of thirty nine monogenic lines (*Lr*'s) against fifteen pathotypes of *P. triticina* under greenhouse condition during 2013/2014 growing season at seedling stage.

Monogenic line (*Lr*'s)	Leaf rust pathotypes / Leaf rust infection type*														
	BBCHS	BCCMS	BJTJG	CJNBD	JFKTS	LCJKB	LMSDB	MHCQQ	NSHLQ	PBBFB	PTQTT	QHJHJ	RJCRQ	SCLKB	SKPSS
1	L**	L	L	L	L	H	H	H	L	H	H	H	H	H	H
2a	L	L	L	L	H	L	L	L	L	L	L	H	H	H	H
2b	H***	H	L	L	H	H	L	L	L	H	H	H	H	H	H
2c	L	L	L	L	H	L	L	L	H	H	H	L	L	H	H
3	L	L	L	H	L	L	L	H	L	H	H	L	H	L	L
3ka	L	L	H	H	L	L	H	L	L	L	H	L	L	H	H
3bg	L	H	L	H	H	L	L	L	L	H	H	L	H	L	H
9	L	L	L	L	L	L	H	L	H	L	H	L	L	L	L
10	L	H	L	L	H	L	L	H	H	L	H	L	H	L	H
11	L	L	H	L	H	H	H	L	H	L	H	H	L	L	L
12	L	H	L	L	H	L	L	H	L	H	H	H	L	L	H
13	L	H	H	L	H	L	H	L	L	H	H	H	H	H	H
14a	L	H	H	H	L	H	H	H	L	L	H	L	H	H	H
14b	H	H	L	L	H	L	L	H	H	L	H	L	H	L	H
15	H	H	H	L	H	L	L	H	H	L	H	H	H	L	H

Table 3. Contd.

16	L	L	H	H	L	L	L	H	H	L	H	H	H	L	H
17	L	L	H	H	H	H	H	L	L	L	L	H	L	L	H
18	H	L	H	L	H	H	L	H	L	L	H	H	H	H	H
19	L	H	L	L	L	L	L	L	L	L	L	L	L	L	L
20	H	L	L	L	H	L	L	L	H	H	L	L	L	H	L
21	L	L	H	L	H	H	H	L	L	H	H	L	L	H	H
22a	L	H	L	L	H	L	L	L	L	L	H	H	H	H	H
22b	H	H	L	H	L	H	L	H	L	L	H	L	H	L	H
23	H	H	H	L	H	L	H	L	H	H	L	H	H	L	H
24	L	L	H	H	H	L	L	L	H	L	H	L	H	L	H
25	H	H	H	L	H	H	L	L	H	L	H	L	L	H	H
26	L	H	L	L	H	H	H	H	L	L	H	H	L	H	H
27	H	L	H	H	H	L	L	L	H	L	L	H	H	H	H
28	H	H	H	L	H	L	L	L	H	H	L	L	H	L	H
29	H	H	H	L	H	H	H	L	H	H	L	H	H	H	H
30	H	H	H	L	H	L	L	H	H	L	L	L	H	L	H
32	H	L	L	L	H	H	L	H	H	L	L	L	L	L	H
33	H	L	L	L	H	L	H	H	H	L	L	H	L	L	H
34	H	H	L	L	H	L	H	L	L	L	L	L	L	L	L
35	H	H	L	H	H	L	L	L	L	L	H	H	L	L	H
36	H	H	L	H	H	L	H	L	H	L	L	H	H	L	L
37	H	H	H	H	H	L	L	H	H	H	H	L	H	H	L
42	L	L	L	L	L	L	L	L	L	L	H	L	L	L	L
B	L	H	L	L	H	L	L	H	L	H	H	H	L	H	H

* Infection type as described by Stakman et al. (1962). ** L= Low infection type (0, 0; ,1 and 2). *** H= High infection type (3 and 4).

Data in Table 6 showed that percentage final rust severity of the tested wheat genotypes compared with the check genotype Sids 1 at adult stage under field conditions during the three tested growing seasons. In 2011/2012 growing season, the wheat genotypes Shandweel 1 (2.00%), Misr 1 (3.00%), Sakha 94 and Sids 13 (each with 4.33%), Giza 168 (5.00%), Misr 2 (6.67%), Sids 12 (8.33%), Sakha 95 (10.00%), Gemmeiza 9 and Gemmeiza 10 (each with 13.33%) were showed the lowest values of final rust severity (did not exceed up to 20%). While, Gemmeiza 11 (26.67%), Gemmeiza 7 (30.00%) and Sids 1 (76.67%) showed higher percentage values of FRS at Nubariya location.

Moreover, the wheat genotype Shandweel 1 showed complete resistance and no visible infection occurred. Also, the wheat genotypes Sids 12, Misr 1, Sids 13, Misr2, Sakha 94, Gemmeiza 10, Giza 168, Gemmeiza 9 and Sakha 95 showed low percentage levels of FRS that is, 3.00, 3.00%, 3.67, 4.33, 4.78, 6.00, 8.33, 10.00 and 13.33%, respectively at Italy El-Baroud location during this season. At Shibin El-Kom location, the wheat genotypes Sids 12, Misr 1, Misr 2 and Shandweel 1 were very resistant and showed no visible infection. Also, the wheat genotypes Sids 13 (2.00%), Sakha 94 (3.67%), Sakha 95, Gemmeiza 9 and Gemmeiza 10 (each with 6.67%), Giza 168 (8.33%) and Gemmeiza

Table 4. Leaf rust resistance genes (*Lr*'s) probably present in the thirteen Egyptian wheat genotypes.

Genotype	Probable *Lr* gene
Sakha 94	+?*
Sakha 95	12, 13, 15, 22a, 26, 35, B +?
Giza 168	26, 29 +?
Gemmeiza 7	+?
Gemmeiza 9	+?
Gemmeiza 10	11, 17, 26, 29 +?
Gemmeiza 11	29 +?
Sids 1	23, 29 +?
Sids 12	15, 23, 25, 28, 29, 30, 37 +?
Sids 13	+?
Misr 1	27, 36, 37 +?
Misr 2	2c, 37 +?
Shandweel 1	25, 29 +?

* +? = Means that the concerned genotype may have additional gene (s)that were not detected using the tested *Lr* genes and isolates of the study.

Table 5. Number of postulated leaf rust resistance genes (*Lr*'s) and their percentage of frequency in thirteen Egyptian wheat genotypes at seedling stage under greenhouse condition during 2013/14 growing season.

Monogenic line (*Lr*'s)	No. of genotype possessing *Lr* gene	Gene frequency (%)
1	0	0.00
2a	0	0.00
2b	0	0.00
2c	1	6.66
3	0	0.00
3ka	0	0.00
3bg	0	0.00
9	0	0.00
10	0	0.00
11	1	6.66
12	1	6.66
13	1	6.66
14a	0	0.00
14b	0	0.00
15	3	20.00
16	0	0.00
17	1	6.66
18	0	0.00
19	0	0.00
20	0	0.00
21	0	0.00
22a	1	6.66
22b	0	0.00
23	3	20.00
24	0	0.00
25	2	13.33
26	3	20.00
27	1	6.66
28	1	6.66

Table 5. Contd.

29	7	46.66
30	1	6.66
32	0	0.00
33	1	6.66
34	0	0.00
35	2	13.33
36	2	13.33
37	3	20.00
42	0	0.00
B	1	6.66

11(11.67%) showed low percentage levels of FRS. While, the wheat genotypes Gemmeiza 7 (26.67%) and Sids 1 (73.33%) showed high percentage levels of FRS.

Data of percentage final leaf rust severity in the second season (2012/2013) showed that the wheat genotypesShandweel 1, Sids 13, Sids 12, Misr 1, Misr 2, Sakha 94, Gemmeiza 11, Gemmeiza 9, Giza 168, Gemmeiza 10 and Sakha 95 showed the lowest percentage values of FRS (less than 20.00%). While the wheat genotypes Gemmeiza 7 and Sids 1 showed the highest percentage values of FRS during this season at the three locations that is, Nubariya, Itay El-Baroud and Shibin El-Kom.

In 2013/2014 growing season, the wheat genotypes Misr 1, Misr 2 and Shandweel 1 showed complete resistance and no visible infection occurred. Also, the wheat genotypes Sids 12 (2.00%), Gemmeiza 12 (4.33%), Sids 13 (6.00%), Sakha 94, Giza 168 and Gemmeiza 9 (each with 6.67%) showed the lowest percentage values of FRS at Nubariya location. While, the wheat genotypes Sakha 95 (23.33%), Gemmeiza 11 (36.67%), Gemmeiza 10 (43.33%), Gemmeiza 7 and Sids 1 (each with 76.67%) showed the highest percentage values of FRS at Nubariya location. The wheat genotypes Shandweel 1, Misr 1, Sids 12, Sids 13, Misr 2, Sakha 94, Gemmeiza 9, Sakha 95, Giza 168 and Gemmeiza 10 showed the lowest percentage values of FRS at the other two tested locations (Itay El-Baroud and Shibin El-Kom) during this season. While, the wheat genotypes Gemmeiza 11, Gemmeiza 9 and Sids 1 showed the highest percentage values of FRS at the two locations Itay El-Baroud and Shibin El-Kom.

Data of mean percentage values of FRS during three seasons and at three locations indicated that the wheat genotypes Shandweel 1 (0.22%), Misr 1 (2.33%), Sids 12 (3.22%), Sids 13 and Misr 2 (each with 3.41%), Sakha 94 (5.36%), Gemmeiza 9 (7.11%) and Giza 168 (8.26%) showed the highest resistance response with the lowest mean percentage values of FRS, followed by Sakha 95 (12.41%), Gemmeiza 10 (14.37%) and Gemmeiza 11 (18.74%). While, the wheat genotypes Gemmeiza 7 (41.48%) and Sids 1 (72.96%) were susceptible and showed the highest mean percentage values of FRS.

The wheat genotype Shandweel 1 showed resistance infection type. While, the wheat genotypes Sids 12, Sids 13, Misr 1 and Misr 2 showed moderately resistance infection type and the reminder genotypes showed susceptible infection type at the three tested locations during the three seasons.

Area under disease progress curve (AUDPC)

Data in Table 7 estimated the mean values of AUDPC over the three years and three locations.There are significant differences in AUDPC means among the tested wheat genotype sat P = 0.05. Meanwhile, no significant differences in means of AUDPC of environments (years and locations) as well as the interaction between genotypes and environments. Moreover, the tested wheat genotypes can be classified into three groups. The first group race-specific resistant includes wheat genotypes of Shandweel 1 (4.67), Misr 1 (37.33), Sids 12 (44.46), Misr 2 (44.98) and Sids 13 (54.05). These genotypes displayed the highest levels of adult plant resistance and showed the lowest values of AUDPC. Also, they showed resistant and moderately resistant infection types.

The second group included the wheat genotypes Sakha 94, Gemmeiza 9, Giza 168, Sakha 95, Gemmeiza 10 and Gemmeiza 11, which they displayed acceptable levels of adult plant resistance. However, results showed low values of AUDPC for these genotypes that is, 57.04, 62.74, 80.24, 102.02, 137.28 and 171.63, respectively. Also, this group showed susceptible infection type. Therefore, they have been classified as slow-rusting or partially resistant genotypes. Whereas, the third group included Gemmeiza 7 (429.07) and Sids 1 (751.85), which they showed the highest values of AUDPC to leaf rust infection, Also, displayed the lowest levels of adult plant resistance and these genotype sclassified as fast-rusting genotypes.

Table 6. Percentage of final rust severity (FRS) of *P. triticina* and infection type on thirteen wheat genotype sunder field conditions at three locations during three growing seasons.

Genotype	Infection type	Season / Location / FRS (%)									Mean FRS (%)
		2011/2012			2012/2013			2013/2014			
		Nubariya	Itay El-Baroud	Shibin El-Kom	Nubariya	Itay El-Baroud	Shibin El-Kom	Nubariya	Itay El-Baroud	Shibin El-Kom	
Sakha 94	S	4.33	4.78	3.67	6.67	7.22	5.00	6.67	5.56	4.33	5.36
Sakha 95	S	10.00	13.33	6.67	16.67	13.33	10.00	23.33	10.00	8.33	12.41
Giza 168	S	5.00	8.33	8.33	6.67	13.33	4.33	6.67	8.33	13.33	8.26
Gemmeiza 7	S	30.00	23.33	26.67	43.33	33.33	23.33	76.67	53.33	63.33	41.48
Gemmeiza 9	S	13.33	10.00	6.67	8.33	4.33	4.33	6.67	6.67	3.67	7.11
Gemmeiza 10	S	13.33	6.00	6.67	16.67	13.33	5.00	43.33	16.67	8.33	14.37
Gemmeiza 11	S	26.67	13.33	11.67	20.00	6.67	3.67	36.67	26.67	23.33	18.74
Sids 1	S	76.67	83.33	73.33	83.33	73.33	63.33	76.67	63.33	63.33	72.96
Sids 12	MR	8.33	3.00	0.00	4.33	2.00	2.00	2.00	4.33	3.00	3.22
Sids 13	MR	4.33	3.67	2.00	2.67	2.00	2.00	6.00	4.33	3.67	3.41
Misr 1	MR	3.00	3.00	0.00	4.33	3.33	2.00	0.00	3.33	2.00	2.33
Misr 2	MR	6.67	4.33	0.00	4.33	4.33	3.00	0.00	3.67	4.33	3.41
Shandweel 1	R	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
L.S.D. of genotypes at 5% =			4.90			15.75			6.37		
L.S.D. of environments (years and location) at 5%=			NS			NS			NS		
L.S.D. of interaction (G × E) at 5% =			NS			NS			NS		

NS= Non-significant at P=0.05.

Table 7. Area under disease progress curve (AUDPC) of *Puccinia triticina* on thirteenwheat genotypes under field conditions at three locations during three growing seasons.

Genotype	Season / Location / AUDPC									Mean AUDPC
	2011/2012			2012/2013			2013/2014			
	Nubariya	Itay El-Baroud	Shibin El-Kom	Nubariya	Itay El-Baroud	Shibin El-Kom	Nubariya	Itay El-Baroud	Shibin El-Kom	
Sakha 94	65.33	65.33	42.00	56.00	56.00	56.00	65.33	65.33	42.00	57.04
Sakha 95	82.83	94.50	65.33	112.00	94.50	94.50	157.50	122.50	94.50	102.02
Giza 168	53.67	94.50	94.50	77.00	82.83	65.33	65.33	94.50	94.50	80.24
Gemmeiza 7	443.33	186.67	326.67	443.33	350.00	338.33	735.00	490.00	548.33	429.07
Gemmeiza 9	82.83	94.50	65.33	56.00	56.00	56.00	56.00	56.00	42.00	62.74
Gemmeiza 10	155.17	65.33	65.33	112.00	94.50	65.33	420.00	163.33	94.50	137.28
Gemmeiza 11	280.00	94.50	65.33	91.00	56.00	42.00	408.33	350.00	157.50	171.63

Table 7. Contd.

Sids 1	840.00	840.00	723.33	805.00	781.67	665.00	781.67	665.00	665.00	751.85
Sids 12	82.83	42.00	0.00	51.33	42.00	42.00	42.00	56.00	42.00	44.46
Sids 13	65.33	42.00	42.00	51.33	42.00	42.00	94.50	65.33	42.00	54.05
Misr 1	42.00	42.00	0.00	56.00	56.00	42.00	0.00	56.00	42.00	37.33
Misr 2	82.83	56.00	0.00	56.00	56.00	42.00	0.00	56.00	56.00	44.98
Shandweel 1	42.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.67
L.S.D. of genotypes at 5% =			50.13		36.63			58.75		
L.S.D. of environments (years and location) at 5% =			NS		NS			NS		
L.S.D. of interaction (G × E) at 5% =			NS		NS			NS		

NS= Non-significant at P=0.05.

DISCUSSION

One hundred eighteen, one hundred sixty-six and sixty-one single isolates of leaf rust were evaluated for virulence during three growing seasons that is, 2011/2012, 2012/2013 and 2013/2014, respectively against the thirteen tested wheat genotypes. During 2011/12 growing season low virulence frequencies of leaf rust have been found on Misr 1, Sids 13, Misr 2, Sids 12 and Sakha 94 wheat genotypes.

In 2012/2013 growing season low virulence frequencies of leaf rust have been found on the wheat genotypes Misr 1, Misr 2 and Sids 12. While, in 2013/2014 growing season the wheat genotype Misr 1 showed low virulence frequency. Occurrence of virulence was stable on the wheat genotype Misr 1 during the three seasons of this study, while, virulence frequencies of Misr 2 and Sids 12 which were almost stable during the two growing seasons 2011/2012 and 2012/2013. Moreover, little changes in varietal resistance against the tested isolates in the growing seasons of study were recorded; this is might be due to the differences in the genetic make-up of the tested wheat genotypes (Negm et al., 2013).

Based on results of gene postulation, five wheat genotypes that is, Sakha 94, Gemmeiza 7, Gemmeiza 9, Gemmeiza 11 and Sids 13 of the thirteen tested genotypes appeared to have either none or only one known *Lr* gene. While, all of the tested wheat genotypes appear to have one or more unidentified leaf rust resistance genes.The wheat genotypes Sakha 95 and genotypes *Lr* 12, *Lr* 13, *Lr* 15, *Lr* 22a, *Lr* 26, *Lr* 35 and *Lr* B in the wheat genotype Sakha 95.*Lr* 15, *Lr* 23, *Lr*25, *Lr*28, *Lr*29, *Lr* 30 and *Lr*37 were detected in the wheat genotype Sids 12. Similar results were recorded by McVey (1989), Youssef (2006), Boulot (2007), Shynbolat and Aralbek (2010) and Abdelbacki et al. (2013).

Changing in race pathogenesis led the breeders to involving alternative forms of resistance that would be more durable such as slow rusting or partial resistance (Broers, 1989; Singh et al., 2000a, b). It has been demonstrated that durable rust resistance is more likely to be of adult plant type rather than of seedling infection type and is not linked with the genes producing hypersensitive reaction (McIntosh, 1992; Bariana et al., 2001). Durable rust resistance is a mechanism conferring resistance to a genotype for long period of time during its widespread cultivation in a favorable environment for a disease (Johnson, 1978, 1988). This type of resistance is mainly associated with the minorgenes, which are also known as slow rusting genes. The concept of slow rusting in wheat was previously recommended by Caldwell (1968).

Many researchers have emphasized the need to identify and exploit durable resistance. Johnson and Law (1975) defined durable resistance as a resistance source that remained effective after widespread deployment over a considerable period. A general concept of a durable resistance source for cereal rusts is that it is polygenic, likely to express at adult plant stage, non-race-specific and produce non-hypersensitive response to infection.

The tested wheat genotypes were evaluated at adult plant stage at three locations that is, Itay El-Baroud and Nubariya Agricultural Research Stations as well as the Farm of the Faculty of Agriculture, Minufiya University, Shibin El-Kom during three successive growing seasons that is,

2011/2012, 2012/2013 and 2013/2014. Percentage of FRS was recorded for each of the tested genotypes. However, the wheat genotype Shandweel 1 was highly resistant and showed lowest percentage level of FRS and resistance infection type. Moreover, the wheat genotypes Sids 12, Sids 13, Misr 1 and Misr 2 were also highly resistant and showed low percentage level of FRS and moderately resistance infection type. Resistance to leaf rust in these wheat genotypes mainly due to race-specific resistance gene (s), which were showed infection type resistance (R) to moderately resistance (MR). German and Kolmer (1992) found that individual major genes for adult plant resistance to leaf rust can show enhancement effectiveness when combined in wheat genotypes. However, the wheat genotypes Sakha 94, Gemmeiza 9, Giza 168, Sakha 95, Gemmeiza 10 and Gemmeiza 11 showed low percentage levels of FRS (did not exceeded up to 20%) also, these genotypes showed susceptible infection type (S). These genotypes displayed an adequate level of partial resistance to leaf rust infection, in comparison with the two genotypes Gemmeiza 7 and Sids 1 (fast rusting or highly susceptible genotypes). These results are previously supported by Bassiony (1979) and Nazim et al. (1983, 1990).

AUDPC is a good indicator of adult plant resistance under field condition (Wang et al., 2005). They added that genotypes which had low AUDPC and terminal severity values may have good level of adult plant resistance (Wang et al., 2005). Furthermore, AUDPC, in particular, is the result of all factors that influence disease development such as differences in environmental conditions, varieties and population of the pathogen (Pandey et al., 1989; Lal Ahmed et al., 2004; Singh et al., 2005; Boulot, 2007).

According to the obtained results and depending on the mean values of AUDPC, the wheat genotypes Sakha 94, Gemmeiza 9, Giza 168, Sakha 95, Gemmeiza 10 and Gemmeiza 11 showed lowest values of AUDPC. These results indicated that such genotypeshave good level of adult plant resistance under field conditions in three locations through three growing seasons to leaf rust and can be used as resistance sources. Therefore, this group of genotypes characterized as partially or slow rusting resistant group. While, the two wheat genotypes Gemmeiza 7 and Sids 1 showed the highest AUDPC values. These genotypes classified as the highly susceptible or fast rusting genotypes group, similarly to those reported by Nazim et al. (1990); Denissen (1993) and Singh et al. (2005).

Conclusion

According to the data obtained, nineteen known leaf rust resistance genes and one or more unknown genes were postulated in the thirteen tested wheat genotypes. These findings should be useful in the Egyptian wheat breeding programs in order to improve leaf rust resistance. Also, the present study revealed that most of the tested wheat genotypes were having enough resistance, ranging from complete resistance to partial resistance. Also, most of the tested wheat genotypes exhibited better performance under high disease pressure shown by susceptible varieties. Further studies for testing stability of the tested wheat genotypes over growing seasons and locations against leaf rust along with other desirable characters must be studied.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Use of kitchen waste based bio-organics for strawberry (*Fragaria x ananassa* Duch) production

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Utilization of waste materials is now getting popularity in agriculture production as a part of organic management. In the present experiment liquid manure from kitchen waste along with animal and plant parts was prepared and utilized them for strawberry (*Fragaria x ananassa* Duch) cv. Sweet Charlie production. The aim of the study was to see the effect of liquid manure on improvement of vegetative growth, yield and quality of strawberry fruits grown in high pH soil (8.2) at Lucknow subtropical condition of Uttar Pradesh, India. Runners of strawberry was planted at the spacing of 30 x 30 cm accommodating 9 plants per plot following randomized block design comprising 10 treatments replicated thrice. The results showed that use of treatment T_7 increased the plant height recorded at different dates after transplanting as compared to other treatments. The treatment T_7 was found to be better for improvement of vegetative growth, recording maximum number of branches per plant, number of leaves per plant, length and breadth of leaves and also caused early flowering (75.33 days after transplanting), maximum number of flowers per plant and maximum yield (fruits/plant and kg/ha). Similarly, good quality fruits having maximum TSS (9.3°B) and ascorbic acid (54.75 mg/100 g) were obtained under treatment T_7, though acidity was increased (0.91%) in this treatment. Thus, it can clearly be concluded that, liquid manure is effective for crop growth, better yield and good quality fruits.

Key words: Liquid manuring, quality, strawberry, yield.

INTRODUCTION

Strawberry *(Fragaria x ananassa* Duch) is one of the most aroma containing, nutritive and delicious fruits. Strawberry plants are spread all over the country for their attractive colour and growing adaptability to wide range of soil and environmental conditions. The genus *Fragaria* belongs to family Rosaceae containing 17 spp., among them strawberry is one of the most popular edible manmade octaploid (2n = 56) hybrid. India has 0.20 m ha area with production of 1.89 mt strawberry fruits and the leading states are Himachal Pradesh (0.50 mt) and Meghalaya (1.00 mt) (NHB Database, 2013-14). Although, it prefers temperate climate for its luxurious population, it also had a good performance under subtropical Lucknow condition. Fructose and glucose are the major sugars found in the strawberry and small proportion of sucrose is also found in strawberry (Giampieri et al., 2012). The red colour of fruits is mainly due to the presence of anthocyanin, pelarogonidin3-monoglucoside and traces

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of cyaniding and it is rich in antioxidants (Meyers et al., 2003). Considering the environmental safety and safe gourd from health hazards caused by synthetic chemical fertilizers and pesticides, people are approaching for organic farming (Maji and Das, 2008; Maji, 2013). Utilization of waste materials is an important part for modeling of sustainable agricultural system (Speelman et al., 2014). Magdoff and Weil (2004) reported that organic matter is the most important substrate for sustainable production as well as management of soil borne diseases while experimenting with chemical fertilizers, biofertilizers and organic manures (Aldahmani et al., 2010; Mir et al., 2013; Muoneke et al., 2014). Strawberry is a nutrient loving crop. Nutrients are very effective component in yield and quality improvement of strawberry fruits (May and Pritts, 1990). The present experiment is objected to supply the organic nutrients directly (foliar spray) to strawberry crop for easy and quick availability of nutrients. Liquid manure was made from common kitchen wastes and easily available local resources. Liquid manures are cost effective and renewable. Bio-fertilizers including liquid manure are known to increase the yield of strawberry (Shiow and Shin, 2002). Habashy and Laila (2005) studied that the plant growth and yield of wheat crop were increased by fertilization with humic acid at a rate of 100 ppm. Foliar application of humic acid up to 6 l/fed linearly increased total yield of watermelon (Salman et al., 2005). Liquid manure and some biodynamic preparations like Panchagavya, Jeevamruth and Beejamruth contain macro nutrients, essential micro nutrients, many vitamins, essential amino acids, growth promoting factors like IAA, GA and also beneficial microorganisms (Palekar, 2006; Sreenivasa et al., 2010). Utilization of agri-food waste was also studied by Serrano et al. (2014) and they found that fish waste along with glycerol increased the treatment capacity of strawberry. Preparation of liquid or solid manures from kitchen waste is not new but it is not popular so much. But, technical and compositional details have not been found so far. It is very useful and effective way for management of kitchen and domestic wastes. Nutritional details or other components present in kitchen waste are not found so far. Keeping the view, the present investigation has been planned to observe the influence of liquid manure on vegetative growth, yield and quality of strawberry fruits.

MATERIALS AND METHODS

Experimental site

The present experiment was held at Horticultural Research Farm (Pragya Vatika), Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India 226025 during 2013- 2014. The soil pH was estimated before the experiment and it was determined as very high pH soil having pH 8.2. The soil type was sandy loam having EC (1:1) 0.26 and available N -110.50 kg ha^{-1}, P_2O_5- 40.5 kg ha^{-1} and K_2O -190.4 kg ha^{-1}.

Agro-climatic conditions

Lucknow is situated in the central part of Uttar Pradesh and comes under sub-tropical drier climate. Maximum temperature is ranging from 29.3 to 47°C in summer and minimum ranging from 3.5 to15°C in winter with relative humidity of 60 to 80% in different season of the year. Nearly 85% of the total annual rainfall (750 mm/annum) is received during the monsoon (only up to the September) with some scattered showers in winter brought by North-East monsoon. During the experimental period, the maximum and minimum temperature was 39.8 and 7.8°C and RH 98 and 25%, respectively. The average temperature and relative humidity was presented in Figure 1.

Planting materials and transplanting

The planting materials of strawberry cv. Sweet Charlie were obtained from Scientific Seedlings India Pvt. Ltd., Pune, Solapur Road, Dist. Pune (Maharashtra)-412202. Runners of strawberry plants were planted at spacing of 30 cm x 30 cm accommodating 9 plants per plot with making a small hole as it is a shallow rotted crop and a light irrigation was given immediate after transplanting just to moisten the soil moisture and maintain physiology of runners.

Preparation and application of liquid manure

The liquid manure was prepared by the fermenting the mixture of cow dung, potato (*Solanum tuberosum*) peels, carrot (*Daucus carota*) peels, legume (*Medicago* sp.) peels, neem (*Azadirachta indica*) leaves, tulsi (*Ocimum sanctum*) leaves in water. These household and kitchen waste materials were mixed into a big container following combination as stated in Table 1. After mixing, these materials were kept for 45 days for fermentation. In these 45 days it was stirred in every three days interval. Prepared liquid manure was filtered and taken few amount as sampling for nutrient status analysis. Nutrient analysis was done for macro and micro nutrients at Soil Testing Lab, Central Soil Salinity Research Institute (CSSRI), Alambagh, Lucknow following a standard procedure after centrifugation and nutrient status was presented in Table 2. The liquid manure was applied in three dilutions that is, 1: 5 (M_1), 1:10 (M_2) and 1:20 (M_3). The treatment combination was prepared as T_1 for control (C_0M_0), T_1 (C_1M_1), T_2 (C_1M_2), T_3 (C_1M_3), T_4 (C_2M_1), T_5 (C_2M_2), T_6 (C_2M_3), T_7 (C_3M_1), T_8 (C_3M_2) and T_9 (C_3M_3).

Statistical analysis

The field experiment was statistically designed with 10 treatments which were replicated three times and laid out under Randomized Block Design. The recorded data were statistically analyzed using analysis of variance at 5% level of significance (Panse and Sukhatme, 1985). Vegetative observations were recorded at 45, 90 and 135 days after transplanting at regular intervals for obtaining the proper results. All fruit morphological characters and quality parameters were studied in the laboratory of Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India following the standard method of AOAC (2000).

RESULTS AND DISCUSSION

Effect of liquid manures on vegetative growth parameters

The experimental findings (Table 3) clearly showed that

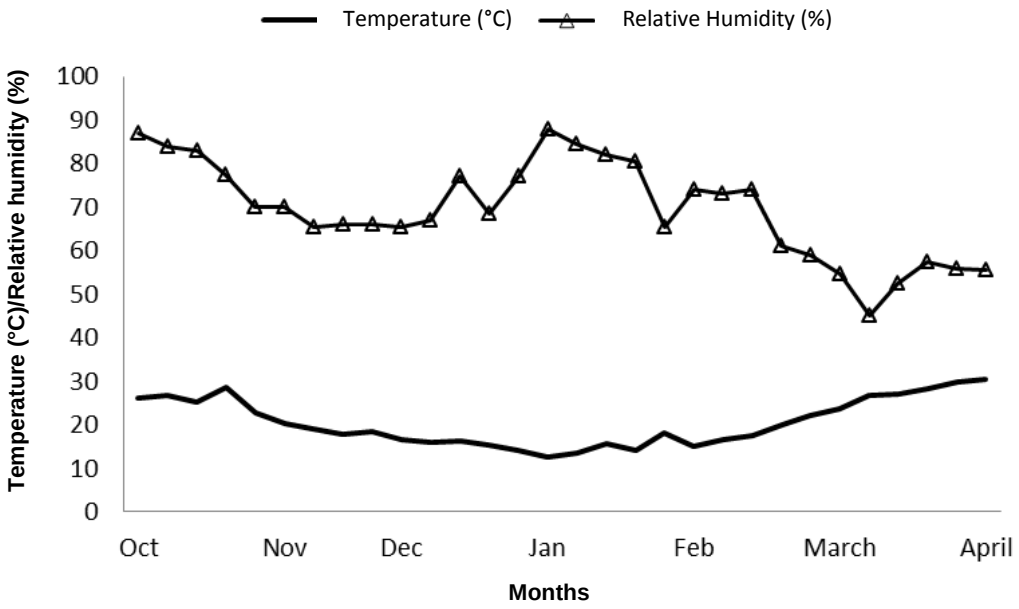

Figure 1. Average temperature and relative humidity status during the experiment.

Table 1. Composition of the different materials of liquid manures.

C_1	Cow dung = 1.0 kg + Potato peels = 750 g + Carrot peels = 400 g + Legume leaves = 400 g + Neem leaves = 200 g + Tulsi leaves = 100 g+ Water = 7 L.
C_2	Cow dung = 1.5 kg + Potato peels = 500 g + Carrot peels = 200 g + Legume leaves = 300 g + Neem leaves = 200 g + Tulsi leaves = 100 g + Water = 7 L.
C_3	Cow dung = 2.0 kg + Potato peels = 200 g + Carrot peels = 200 g + Legume leaves = 300 g + Neem leaves = 100 g + Tulsi leaves = 100 g + Water = 7 L.

Table 2. Nutrient status of various liquid manure compositions.

Treatments	N (%)	P (%)	K (%)	S (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Ca (mg kg^{-1})
T_1	0.13	0.052	0.038	4.1	0.043	0.083	0.018	2.3
T_2	0.081	0.018	0.034	1.38	0.018	0.039	0.01	1.04
T_3	0.046	0.01	0.019	0.16	0.012	0.023	0.006	0.58
T_4	0.154	0.073	0.041	2.1	0.024	0.061	0.050	1.58
T_5	0.096	0.028	0.023	1.06	0.006	0.037	0.023	0.86
T_6	0.051	0.011	0.013	0.58	0.002	0.61	0.013	0.43
T_7	0.188	0.084	0.069	2.18	0.031	0.068	0.071	1.43
T_8	0.102	0.031	0.020	1.17	0.008	0.03	0.025	0.88
T_9	0.067	0.014	0.008	0.71	0.005	0.01	0.017	0.47

application of liquid manures had positive effect on vegetative growth of strawberry. It was noted that the average plant height was significantly improved by application of liquid manure over control. The maximum plant height of 11.8 cm, 12.5 cm, 13.8 cm at 45 DAT, 90 DAT, 135 DAT, respectively was noted with the application of liquid manure (C_3M_1) T_7 closely followed by liquid manure (C_2M_1) T_4 and liquid manure (C_1M_1) T_1. This increase in plant height might be attributed to the fact that presence of higher amount of nitrogen in T_7

Table 3. Effect of liquid manuring on vegetative growth of strawberry.

Treatments	Plant height (cm)			Branch plant $^{-1}$	Number of leaves plant $^{-1}$			Leaf length (cm)			Leaf breadth (cm)		
	45 DAT	90 DAT	135 DAT		45 DAT	90 DAT	135 DAT	45 DAT	90 DAT	135 DAT	45 DAT	90 DAT	135 DAT
T_0	8.1	10.0	10.8	5.75	15	16.67	22	3.4	3.4	3.6	3.4	3.41	3.61
T_1	10.9	11.6	13.3	7.74	18.33	22.7	28.33	4.4	4.9	5.3	3.6	4.9	5
T_2	10.6	11.1	12.6	7.00	17.67	21	29.67	4.1	4.5	4.9	4.1	4.3	4.9
T_3	9.8	10.5	11.5	6.5	17	21.67	27	3.5	4.4	4.5	3.9	4.1	4.3
T_4	10.9	11.8	13.5	8.18	18.33	24.67	27.67	4.5	5.1	5.5	4.5	4.9	5.2
T_5	10.9	11.1	12.7	6.66	13.67	18.33	27.67	4.3	4.4	5	4	4	4.5
T_6	10.3	11.2	12.5	6.55	15.67	19	28.33	3.5	4.5	4.6	3.7	3.8	4.1
T_7	11.8	12.5	13.8	8.21	19.33	24.67	29.33	4.5	5.2	5.8	4.5	4.8	5.5
T_8	10.4	11.6	12.4	7.62	16.33	21	25.67	4.2	4.6	5	4	4.1	4.2
T_9	9.9	10.7	13.1	7.10	17	20	24.33	3.8	4.5	4.7	4.2	4.3	4.7
SEm (±)	**0.576**	**0.565**	**0.742**	**0.721**	**0.984**	**1.217**	**1.480**	**0.209**	**0.208**	**0.255**	**0.222**	**0.201**	**0.273**
CD (P = 0.05)	**1.21**	**1.18**	**1.56**	**1.51**	**2.06**	**2.55**	**3.11**	**0.44**	**0.43**	**0.53**	**0.46**	**0.42**	**0.57**

(nutrient composition of various liquid manure had been presented in Table 2). It is established that nitrogen is the builder of protein and is the main constituent of proto-plasm in plants. Thus, the increase in nitrogen supply accelerated synthesis of amino acids which might have indirectly exhibited increase the vegetative growth in terms of plant height of strawberry plants (Maji and Ghosh, 2006).

Data recorded on number of branches per plant as compared with control showed that the maximum number of branches per plant (8.21) was recorded with the application of liquid manure (C_3M_1) T_7 followed by application of liquid manure (C_2M_1) T_4 and (C_1M_1) T_1. This significant and positive increment in number of branches might be due to the application of liquid manure (C_3M_1) T_7 which resulted more photo synthetic efficiency and growth substances like auxins which favoured the initiation of cell division, cell elongation for growth. The results are similar to the finding of Shwetha (2008) who conducted an experiment to know the effect of nutrient management through organics in soybean wheat cropping system and they reported that significantly higher plant height, number of branches per plant were recorded with the application of organic manures in combination with fermented organics *viz.,* Beejamrut, Jeevamrut, Panchagavya over organics application alone.

The maximum number of leaves per plant (8.21) was recorded with the application of liquid manure (C_3M_1) T_7, followed by application of liquid manure (C_2M_1) T_4. This significant and positive increment in number of leaves under application of liquid manure (C_3M_1) T_7 might be due to the fact that T_7 resulted to more photo-synthetic efficiency favouring the initiation and extension of leaves. Similarly, Sabarad et al., (2004) also stated that the application of organic manures (vermicompost) on banana cv. Rajapuri (Musa AAB) recorded increased plant height, plant girth, number of leaves and number of suckers per plant.

The experimental finding advocated that the average length and width of leaves were also significantly improved by application of liquid manure. The maximum length and width of leaf (4.5, 5.2 and 5.8 cm) and (4.5, 5.2 and 5.8 cm) at 45 DAT, 90 DAT and 135 DAT respectively, were noted with use of liquid manure T_7 closely followed by liquid manure T_4 and T_1. This increase in average length and width of leaves might be attributed to the fact that presence of higher amount of nitrogen in T_7 (as presented in Table 2) which improved and enhanced vegetative growth of crop.

Effect of liquid manuring on flowering and fruiting

Among liquid manures, the application of liquid manure (C_3M_1) T_7 recorded higher number of flowers per plant (24.33) significantly, followed by the application of liquid manure (C_1M_1) T_1 (22.67) (Table 4). The result also found the similarity with the findings of Naidu et al., (2001) who tested that

Table 4. Effect of liquid manuring on flowering, fruiting, yield and fruit quality of strawberry.

Treatments	No. of flowers plant $^{-1}$	Days taken to first flowering	Days taken for first fruiting	Number of fruit plant $^{-1}$	Fruit length (cm)	Fruit diameter (cm)	Fruit volume (ml)	Fruit weight (g)	Fruit yield plant $^{-1}$ (g)	Fruit yield hectare $^{-1}$ (kg)	Ascorbic acid (mg 100g $^{-1}$)	TSS (^{o}Brix)	Acidity (%)
T_0	17.33	96.3	108.5	12.31	3.56	2.09	7.5	10.34	127.3	425.33	53.03	7.33	0.82
T_1	22.67	77.6	91.26	13.5	4.49	3.79	12.5	16.45	222.9	742.93	53.75	8.67	0.83
T_2	18.67	84.5	94.67	12.65	4	3.45	16	14.22	179.9	599.9	53.53	8.33	0.85
T_3	15.67	92.16	106.33	11.16	3.5	3.2	7.5	13.55	150.7	502.63	53.06	7.67	0.89
T_4	20.33	76.01	93.36	13.20	4.75	3.9	15.5	20.21	266.8	889.33	53.96	8.79	0.84
T_5	18.00	86.67	99.66	11.98	4.12	3.41	12.16	15.89	190.5	635.2	53.26	8.31	0.86
T_6	16.67	94.66	105.13	9.99	3.30	3.10	12.5	13.65	136.4	454.9	53.16	7.43	0.90
T_7	24.33	75.33	88.63	13.43	4.9	4.35	17.16	21	282.2	940.67	54.75	9.3	0.91
T_8	18.33	88.57	96.13	13.66	4.3	3.44	12.50	14.45	197.5	658.30	53.62	8.63	0.85
T_9	17.67	95.66	102.80	12.32	3.5	3.1	12.16	14.31	176.4	588.00	53.24	7.57	0.88
SEm (±)	**1.144**	**1.702**	**1.991**	**1.091**	**0.263**	**0.239**	**1.116**	**1.073**	**17.157**	**36.767**	**0.369**	**0.523**	**0.026**
CD (P = 0.05)	**2.40**	**3.57**	**4.18**	**2.29**	**0.55**	**0.50**	**2.34**	**2.25**	**36.05**	**77.24**	**0.77**	**1.09**	**0.05**

application of (100 kg) N + (50 kg) P_2O_5 + (20 t) FYM per ha was significantly superior than the other combinations and gave maximum plant height number of leaves, number of branches as well as number of flower cluster, number of fruits and fruit yield per plant.

It was also observed that the treatments caused early flowering by reducing the requirement of days for first flowering. The minimum number of days (75.33) taken for first flowering was recorded with the application of liquid manure (C_3M_1) T_7 followed by T_4 (C_2M_1), T_1 (C_1M_1), and T_2 (C_1M_2). It was also seen that the treatments were significantly showed earlier fruiting in comparison to control. The minimum number of days (88.63 days) was taken for first fruiting under the application of liquid manure (C_3M_1) T_7 which was closely followed by application of treatment T_4 (91.26 days). The result corroborated with the finding of Arancon et al., (2003) who reported that application of vermicompost in strawberry (*Fragaria* sp.) increased leaf area, number of suckers, number of flowers and shoots per plant. Similar result on flowering and fruiting due to more use of nitrogen was also found by Maji and Ghosh (2007) on pummelo while experimenting on the effect of nitrogen levels on flowering and fruiting.

Effect of liquid manuring on the yield and yield attributing characters

The maximum fruit yield (282.2 g plant^{-1}, 940.64 kg ha $^{-1}$) was recorded with the application of liquid manure (C_3M_1) T_7 and the lowest yield (127.3 g plant $^{-1}$, 425.33 kg ha^{-1}) per plant was recorded in control (Table 4). However, the highest number of fruits per plant (13.66) was found in T_8 but, maximum yield was recorded in T_7. The similar trend was also observed by Veerabhadraiah and Badrinath (2006). They showed that application of Angara and Amrithpani to soil and Panchagavya as foliar spray was found to increase sorghum grain and dry fodder yields. Increase in yield by FYM in strawberry cv. Douglas was also reported by Ilgn et al*.,* (2006).

Effect of liquid manuring on morphology of fruits

The data (Table 4) also revealed that application of liquid manures influenced the fruit length and diameter of strawberry significantly. The highest fruit length and diameter (4.9 and 4.35 cm, respectively) was recorded with liquid manure (C_3M_1) T_7 followed by liquid manure composition (C_2M_1) T_4 (4.75 and 4.35 cm, respectively). The highest fruit volume (17.16 ml) was also recorded with liquid manure (C_3M_2) T_7 followed by T_4 (15.5 ml) and the lowest fruit volume was recorded with liquid manure (C_1M_2) T_2 (16 ml). The treatment T_7 also recorded the highest fruit weight (21 g) was

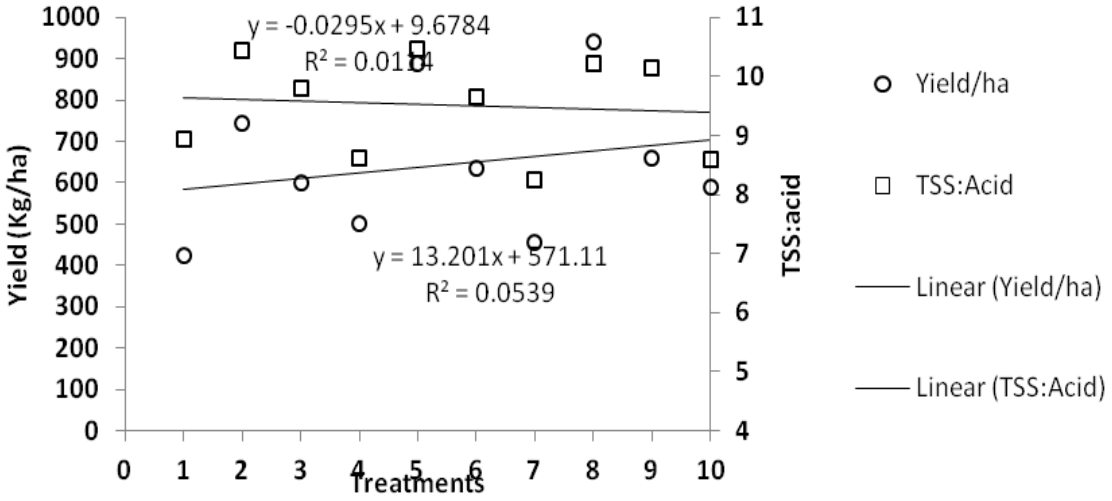

Figure 2. A positive correlation between fruit yield and TSS:acid ratio due to application of liquid manures.

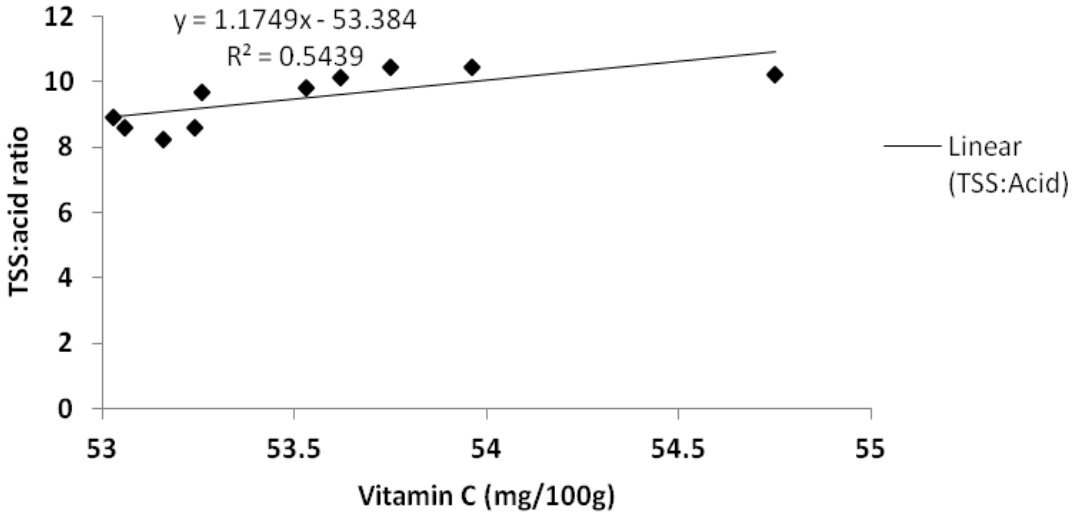

Figure 3. Positive relation between Vitamin C and TSS: acid ratio of strawberry as influenced by liquid manure application.

followed by liquid manure (C_2M_1) T_4 (20.21 g). The fruit weight differed significantly by the application of liquid manures and the lowest fruit weight (10.34 g) was recorded with control (T_0). No literature was found regarding effect of liquid manure on fruit morphological characters, however, Arancon et al*.,* (2004) reported that application of vermicompost on strawberry cv. Chandler exhibited the highest yield, fruit weight and width. Improvement of fruit physical characters with application of liquid manures might be due to the presence of both macro and micro nutrients in liquid manures under study. Positive role of nitrogen on improvement of fruit size and weight was also reported by Maji and Ghosh (2007) in pummelo and Maji and Das (2008) in guava.

Effect of liquid manuring on fruit chemical characters

It is evident from the data that all the treatments significantly increased vitamin C content and thus, maximum (54.75 mg 100 g^{-1}) was recorded under treatment T_7 followed by liquid manure (C_2M_1) T_4 (53.96 mg 100 g^{-1}) and minimum value (53.03 mg 100g^{-1}) was observed in control (T_o). Total soluble solids (TSS) was recorded maximum (9.3°B) in strawberry under treatment T_7 whereas, minimum TSS (7.33°B) of strawberry was observed in control (T_0) (Table 4).

Similarly, quality improvement by use of Panchagavya was also reported by Hannah et al., (2005) and Sharma (2004) who observed the improvement of fruit quality of

banana with spraying of panchagavya solution at 3%. Likewise, TSS and vitamin C application of liquid manures increased titratable acidity also which might affect the TSS: acid ratio. Figure 2 also showed that there was a positive correlation between the liquid manures and fruit yield. Yield was improved from control (T_o) to T_7 and decreased thereafter at T_8. While, negative correlation was observed in case of TSS: acid ratio, though T_7 recorded maximum amount of TSS: acid ratio. A positive correlation was found between vitamin C and TSS: acid ratio (Figure 3). It might be due to the fact that as T_7 counted higher amount of nitrogen (Table 2) and nitrogen has position correlation with acidity of fruits (Maji and Ghosh, 2007) after a certain level. In general, liquid manures had good amount of N, P, K along with S, Zn, Fe, Mn, Ca (Table 2) which might improve the fruit quality in the present investigation.

Conclusion

The present experiment showed that vegetative growth, yield and physico-chemical characteristics of fruits were improved by liquid manure application. Among the treatments T_7 (C_3M_1) showed its potentiality to overall improvement of strawberry production. Therefore, it may be concluded that liquid manure composition (C_3M_1) T_7 is beneficial for strawberry production for its growth, yield and fruit quality.

Conflict of Interests

The authors have not declared any conflict of interests.

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Effect of sowing dates and nitrogen levels for ethanol production from sweet sorghum stalks and grains

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Sweet sorghum is being investigated as a feedstock for ethanol production in semi-arid countries. It can be grown with fewer inputs than other energy crops. In Iran, due to long growing season, ethanol is produced from both sweet sorghum stem and grain. In other countries, ethanol is not produced from sweet sorghum grain due to low grain yield or short growing season. The purpose of this study was to maximize ethanol production from both stem and grain of sweet sorghum. Four planting dates (July 5, July 14, July 25 and August 4) and three nitrogen levels (50, 100 and 150 kg/ha urea) were assigned to the main and subplots, respectively. Plants were harvested at physiological maturity. Stem measurements were: stalk height, stalk diameter, stalk fresh yield, brix value, sucrose content, total sugar and ethanol yield and grain measurements were: number of grain per panicle, 1000 grain weight, grain yield, biological yield, harvest index and ethanol yield. Stem measurements were highest in July 5 planting and lowest in August 4 planting, while grain measurements were highest and lowest in August 4 and July 5 plantings, respectively. Stem and grain measurements in July 15 and July 25 were intermediate. More grain yield, 1000 grain weight and number of grain/panicle were obtained with the application of 150 kg/ha urea than the other two nitrogen levels. Based on the results, in order to obtain the highest ethanol yield from both stem and grain, it is suggested to plant sweet sorghum as early as possible and apply 150 kg/ha urea.

Key words: Sweet sorghum, temperature, planting date, ethanol production, nitrogen levels.

INTRODUCTION

Biofuels have been promoted for reducing greenhouse gas (GHG) emissions and petroleum fuel consumption. Bioethanol is now the dominant biofuel used in transportation sector (Hao et al., 2013). The feedstock that could be used for ethanol production includes:starch and sugar based crops, as well as crop residues (corn stover, wheat straw, rice straw, and sugarcane straw, among others), dedicated energy crops (for example, switchgrass,miscanthus,mixed prairie grasses, and short-rotation trees), and forest residues. Among them, sweet

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Table 1. Average of some meteorological parameters on growth period Sweet sorghum (2012).

Month / Meteorological parameters	July	August	September	October	November
Average temperatures C°	37.1	43.25	43.75	33.5	27.3
maximum temperature C°	43.3	47.5	54.5	39.3	33.2
Minimum Temperature C°	31.0	39.0	33.0	27.7	21.4
Rainfall (mm)	0	0	0	0	0
Average relative humidity (%)	23.4	27.0	28.5	30.0	36.3

sorghum (*Sorghum bicolor* L.) Moench) has emerged as a potential feedstock candidate for bioethanol production because of its date is an important agronomic practice for production of ethanol from sweet sorghum. Optimum planting dates may vary by region. Improper planting date at adverse climatic condition could alter both vegetative and reproduction growth stages. Early planting increases biomass but flowering, pollination and grain filling may be affected by hot summer temperature. Late planting reduces biomass, early flowering, prolonging grain filling and damage due to the cold autumn weather. The biomass yield response of sweet sorghum to planting date is well established. Late planting significantly decreased stalk yield, brix value, sucrose content and days to flowering (Almodares et al., 1994).

Due to favorable environmental conditions during the early growing season, higher sweet sorghum grain (2483 kg ha^{-1}) and millable cane yield (37.17 t ha^{-1}) was achieved (Poornima et al., 2008). Sweet sorghum needs enough nitrogen for its full growth and development. But its response to applied N varied with location. Nitrogen rate had no effect on fermentable sugar yield (Smith and Buxton, 1993), total and stalk dry matter yield at harvest (Barbanti et al., 2006), or fermentable carbohydrate and ethanol yield (Lueschen et al., 1991). In Louisiana, biomass yield has been shown to increase by 140% with application of low water demand, short growing period, sugar and biomass yield potential in less desirable conditions, such as semi-arid and salty lands (Zegada-Lizarazu and Monti, 2012). Sweet sorghum has gained a lot of attention because it possesses numerous characteristics that makes it an appealing bioenergy crop. As a bioenergy crop, sweet sorghum could be used to provide grain starch for hydrolysis, stem juice for direct fermentation, and bagasse as cellulosic feedstock for fermentation or boiler fuel (Saballos, 2008).

In planting of 100 kg ha^{-1} N, yield was not increased with an additional 100 kg ha^{-1} N (Ricaud and Arenneaux, 1990). In the same study, total sugar yield was increased by 150% by applying 100 kg ha^{-1} N, with an increase of only 4% from an additional 100 kg ha^{-1} N. Tropical environments differ from temperate ones in both phenology and yield. Both photoperiod and temperature interact, thus further influencing yield especially for very late plantings (Rao et al., 2013). Much information on the impact of planting date on stalk and sugar yields are available on sweet sorghum genotypes grown in temperate climatic conditions (Han et al., 2012; Burks et al., 2013), but information is very limited on mid-summer planting on ethanol production. The present experiment was conducted to identify optimum time of sowing and N level for achieving higher biomass and grain yield for ethanol production.

MATERIALS AND METHODS

The investigation was carried out at sugar cane research station in Ahwaz, Iran during summer 2012. The station is located at an altitude of 8.8 m above mean sea level and it is intersected 48° 22'E latitude and 31° 5' longitude. The weather is very hot and dry from May to October where maximum temperature could reach to 54.5°C in September (Table 1). The duration of sunshine hours is shown in Table 2. It was from 14 h in July to 10 h in November. The treatments consisted of four planting dates that is, July 5, July 15, July 25 and August 4 and three nitrogen levels, that is 50, 100 and 150 kg urea/ha. Urea was applied in two equal doses; at planting and at 6 to 8 leaf stage. The experiment was laid out in a split plot design with three replications, keeping planting dates as main plots and amount of nitrogen as subplots. Individual subplots measured 3.0 m in width and 7.0 m in length. Before planting 60 kg of triple phosphate and 50% of N were applied as basal. The remaining 50% of N was applied when the plants were at 6 to 8 leaf stage. Sweet sorghum cultivar "Sofra" were hand sown at the above dates as the usual method of sowing on one side of ridges (75 × 15 cm) and the plots were irrigated according to local practices. The other agronomic practices were kept normal and uniform for all the treatments. Plants reached physiological maturity after 120 days for the first three planting dates and 107 days for the fourth planting date. At that stage, plants from 1.5 m long of one central row of each treatment were harvested and growth parameters including plant height and plant diameter were measured. Then the plants were weighed and sub-sample of plants were dried for further analysis. Plants from other 1.5 m long of one central row of each treatment were harvested and total fresh weight (leaves, stalks, and panicles) were determined. The leaves along with sheath were stripped. Panicle with the last inner node (peduncle) was separated and fresh stem weight was estimated. Then, fresh stem were passed through three-roller sugar cane mill.

For measuring brix and sucrose content, Juice was sent to the nearby sugarcane factory. Sucrose content of the juice was obtained from the pol reading and uncorrected brix values (Varma, 1988). Total sugar content was calculated based on its correlation to brix degree values using the following equation as estimated by

Table 2. Sunshines hours from planting to harvest.

Month	Sunshine (Hours and minutes)	Month	Sunshine (Hours and minutes)	Month	Sunshine (Hours and minutes)	Month	Sunshine (Hours and minutes)	Month	Sunshine (Hours and minutes)
June21	14.11	Jily22	13.51	Aug.22	13.04	Sep.22	12.07	Oct.24	11.11
22	14.11	23	13.49	23	13.02	23	12.05	25	11.09
23	14.11	24	13.48	24	13.00	24	12.03	26	11.07
24	14.11	25	13.47	25	12.59	25	12.01	27	11.06
25	14.11	26	13.46	26	13.57	26	11.59	28	11.04
26	14.10	27	13.45	27	12.55	27	11.57	29	11.03
27	14.10	28	13.43	28	12.53	28	11.56	30	11.01
28	14.10	29	13.42	29	12.51	29	11.53	31	10.59
29	14.09	30	13.40	30	12.50	30	11.51	Nov.1	10.57
30	14.09	31	13.39	31	12.49	Oct.1	11.5	2	10.55
July1	14.08	Aug.1	13.38	Sep.1	12.48	2	11.48	3	10.54
2	14.08	2	13.36	2	12.46	3	11.45	4	10.52
3	14.08	3	13.34	3	12.42	4	11.44	5	10.51
4	14.07	4	13.33	4	12.41	5	11.42	7	10.49
5	14.07	5	13.32	5	12.38	6	11.41	8	10.47
6	14.06	6	13.31	6	12.37	7	11.38	9	10.45
7	14.05	7	13.28	7	12.35	8	11.36	10	10.45
8	14.04	8	13.27	8	12.34	9	11.35	11	10.43
9	14.04	9	13.26	9	12.33	10	11.33	12	10.41
10	14.03	10	13.24	10	12.31	11	11.31	13	10.40
11	14.02	11	13.22	11	12.28	12	11.29	14	10.38
12	14.01	12	13.21	12	12.25	13	11.27	15	10.36
13	14.00	13	13.20	13	12.23	14	11.25	16	10.35
14	13.59	14	13.19	14	12.21	15	11.23	17	10.34
15	13.58	15	13.18	15	12.19	16	11.21	18	1033
16	13.58	16	13.16	16	12.18	18	11.2	19	10.31
17	13.57	17	13.14	17	12.16	19	11.18	20	10.30
18	13.55	18	13.11	18	12.14	20	11.17	21	10.28
19	13.54	19	13.09	19	12.12	21	11.15	22	10.26
20	13.53	20	13.08	20	12.1	22	11.13	23	10.24
21	13.52	21	13.05	21	12.09	23	11.12	-	-

Liu and Shen (2008). Total sugar content (%) = [0.8111 × Brix (%)]−0.3728. For calculation of theoretical ethanol production from sweet sorghum fresh biomass, the equations reported by Sakellariou et al. (2007) and Zhao et al. (2009) were modified as follows: total ethanol yield (L ha^{-1}) = total sugar content (%) fresh biomass (Mg ha^{-1}) × 6.5 (conversion factor of ethanol from sugar) × 0.85 (process efficiency of ethanol from sugar) × 1.27(1.00/0.79) (specific gravity of ethanol; gm L^{-1}). Ethanol production from grain sorghum was calculated (ethanol conversion factor, 2.70 gallons per bushel) using USDA report (2006). Biological yield was determined based on the dry samples. The dried panicles were, threshed, weighed and grain yield and its components (No. of grain/panicle, 1000 gr grain weight, and grain weight) were measured. Harvest index was calculated based on the grain yield divided by the biological yield multiplied by 100. Data were analyzed using the SPSS package statistical computer program. The Duncan multiple range test (< 0.05) was used to compare the means.

RESULTS

Stalk plant height, plant diameter, total fresh weight, and stem fresh weight

Sum squares for stem height, stem diameter, fresh weight and stalk fresh weight of four planting dates and three nitrogen levels are presented in Table 3. The effect of planting dates on stem height, stem diameter and total fresh weight were significant at 5% level and for stem fresh weight at 1% level. Mean comparisons for the aforementioned measurements are presented in Table 4. Significant decreases in plant height and plant diameter, total fresh weight and stem fresh weight were found in sweet sorghum planted in August than other planting

Table 3. Sum squares for four planting dates and three nitrogen levels for characteristics measured.

Source of variance	df	Stem height	Stem diameter	Total fresh weight	Stem fresh weight
Block	3	23.30	4.11	8.73	8.33
Planting date (D)	3	2212.86*	55.67*	90.21*	194.57**
Error a	6	172.63	3.47	4.81	3.28
Nitrogen level (N)	2	877.51**	8.37**	15.52	4.33
D x N	6	68.22	0.094	10.67	12.93
Error b	16	71.78	0.50	13.43	15.94
CV%		4.37	4.97	10.32	14.10

**, * significant at 1 and 5 percent level, respectively

Table 4. Mean comparisons* of stem height, stem diameter, total fresh weight and stem fresh weight at four planting dates and three nitrogen levels.

Treatments	Stem Height (cm)	Stem diameter (cm)	Total fresh weight (Kg/ha)	Stem fresh weight (Kg/ha)
Planting dates				
July 5	205.60^{a}	17.60^{a}	37590^{a}	32140^{a}
July 15	189.00^{b}	14.30^{b}	36710^{a}	29790^{a}
July 25	186.20^{b}	13.00^{c}	36880^{a}	29750^{a}
August 4	167.00^{c}	11.80^{c}	30780^{b}	21530^{b}
Nitrogen levels (kg/ha)				
50	184.30^{b}	13.14^{b}	35090^{a}	28450^{a}
100	192.77^{ab}	14.08^{ab}	34600^{a}	27640^{a}
150	203.72^{a}	15.07^{a}	36770^{a}	28810^{a}

*Values within a column followed by same letter are not significantly different at 5% using Duncan Multiple Range Test.

dates (Table 4). Plant height and plant diameter gradually decreased from the first planting date in July 5, by 205 cm height and 17.60 cm in diameter to the last planting date in August which was 167 cm in height and 11.80 cm in diameter. Total fresh weight was not significantly different in the first there planting dates and on average it was 37060 kg/ha. They were significantly higher than August planting which was 30780 kg/ha. Similarly, stem fresh weight was not significant for the first three planting dates. The average stem weight for the first three planting dates was 30560 kg/ha. August 4 planting had significantly lowest stem weight (21530 kg/ha) than the other planting dates. The effect of nitrogen levels on stem height and stem diameter were significant at 1%. Nitrogen increased stem height and stem diameter. They were lowest with the application of 50 kg/ha urea and highest with 150 kg/ha urea. Stem height was 184.30 cm with the application of 50 kg/ha urea and increased to 203.72 cm with 150 kg/ha urea. Similarly, stem diameter was 13.1 cm with the application of 50 kg/ha urea and increased to 15.07 cm with the application of 150kg/ha urea. Plant height and plant diameter with the application of 100 kg/ha urea was not significantly different between application of 50 or 150 kg/ha urea.

Stalk brix, total sugar, sucrose, purity and ethanol yield

Sum squares for brix value, sucrose content, total sugar, juice purity and ethanol yield are presented in Table 5. The effect of planting dates on sucrose content, juice purity and ethanol yield was significant at 1% level and for Brix value and total sugar at 5% level. Mean comparisons for the aforementioned measurements are presented in Table 6. Lowest and highest brix value, sucrose, total sugar, purity and ethanol yield was obtained in early planting (July 5) than late planting (August 4). Brix value was 16.13% in July 5 planting and reduced to 12.50% in August planting. Total sugar decreased from 13.10% to 9.83% as planting date delayed from July 5 to August 4. Sucrose content had similar pattern as total sugar. It was 12.10% in July 5 and 6.71% in August 4. Purity reduced considerably from July

Table 5. Sum squares for four planting dates and three nitrogen levels for characteristics measured.

Source of variance	df	Brix	Sucrose	Total sugar	Juice purity	Ethanol
Block	2	4.46	2.25	2.93	17.97	171599
Planting date (D)	3	32.71*	30.78**	21.52*	691.83**	3717037**
Error a	6	2.18	1.29	1.43	21.16	124111
Nitrogen level (N)	2	0.33	0.02	0.22	1.35	71932
D × N	6	1.32	0.94	0.87	22.68	154372
Error b	16	0.47	0.32	0.31	10.44	144819
CV%		4.69	11.63	4.73	4.82	15.9

**, *significant at 1 and 5 percent level, respectively.

Table 6. Mean comparisons* for brix, total sugar, sucrose content , purity and ethanol yield at four planting dates and three nitrogen levels.

Treatments	Brix %	Total sugar %	Sucrose %	Purity %	Ethanol yield l/ha
Planting dates					
July 5	16.13^{a}	13.10^{a}	12.10^{a}	74.15^{a}	2864.56^{a}
July 15	15.93^{a}	13.04^{a}	11.74^{a}	72.48^{a}	2622.90^{b}
July 25	14.57^{b}	11.44^{b}	9.47^{b}	66.65^{b}	2389.11^{c}
August 4	12.50^{c}	9.83^{c}	6.71^{c}	54.78^{c}	1475.35^{d}
Nitrogen levels (kg/ha)					
50	15.21^{a}	11.76^{a}	9.94^{a}	67.40^{a}	3130.19^{a}
100	14.34^{a}	11.78^{a}	9.23^{a}	66.81^{a}	3046.09^{a}
150	14.78^{a}	12.00^{a}	10.06^{a}	66.82^{a}	3345.03^{a}

*Values within a column followed by same letter are not significantly different at 5% using Duncan Multiple Range Test.

5 planting to August 4 planting. It was 74.15 and 54.78% in July 5 and August 4, respectively. Delay in planting considerably had effects on ethanol yield. It was significantly reduced from July 5 planting to August 4 planting. It was 2864.56 l/ha in July 5 planting and it reduced considerately to 1475.35 l/ha in August 4 planting.

Correlation coefficient

Correlation coefficient among characteristics measured is shown in Table 7. Ethanol yield was significantly correlated with stem height, stem diameter, stem fresh weight, total fresh weight, brix value, sucrose content, total sugar and purity. Delayed planting date from July 5 to August 4 reduced stem diameter, stem height, total fresh weight and stem fresh weight considerably (Table 4). So in order to increase ethanol yield, sweet sorghum should be planted early in the season. Also other characteristics such as brix value, sucrose content, total sugar and purity decreased by late planting (Table 6). Thus in order to achieve the highest ethanol yield, sweet sorghum should be planted as early as temperature or following wheat harvest in Kuzestan province.

Grain and its components

Sum squares for grain yield, 1000 grain weight, number of grain/panicle, biological yield, harvest index and ethanol yield is presented in Table 8. The effect of planting dates on grain yield, biological yield, harvest index and ethanol yield was significant at 1% level and for 1000 grain weight and number of grain/panicle at 5% level. Mean comparisons for the above measurements are presented in Table 9. Grain weight, 1000 grain weight, number of grain/panicle, harvest index and ethanol yield were lowest in July 5 planting and highest in August planting. But biological yield was highest in July 5 planting and lowest in August 5 planting. The afore-mentioned measurements were intermediate in July 15 and July 25 plantings. Grain yield increased from 1437.73 kg/ha in July 5 planting to 2411.92 in August planting. Number of grain/panicle increased from 1195.11 in July 4 planting to 1457.57 in August planting. 1000 grain weight was 10.91 and 15.07 for July 5 planting and August 4 plantings, respectively. Ethanol yield was 619.70 l/ha in

Table 7. Correlation coefficient among characteristics measured.

Trait	Stem height	Stem diameter	Stem fresh weight	Total fresh weight	Brix value	Sucrose content	Total sugar content	Purity %	Ethanol yield
Stem height	1								
Stem diameter	0.898**	1							
stem fresh weight	0.733**	0.731**	1						
total fresh weight	0.746**	0.712**	0.764**	1					
Brix value	0.726**	0.749**	0.791**	0.987**	1				
Sucrose content	0.746**	0.712**	0.764**	1.000 **	0.987**	1			
Total sugar content	0.712**	0.756**	0.752**	0.968**	0.991**	0.968**	1		
Purity %	0.781**	0.765**	0.960**	0.910**	0.920**	0.910**	0.883**	1	
Ethanol yield	0.698*	0.630*	0.962**	0.673*	0.675*	0.673*	0.620*	0.902**	1

**Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.

Table 8. Sum squares for four planting dates and three nitrogen levels for characteristics measured.

Source of variance	df	Grain yield	1000 grain weight	Number of grain/panicle	Biological yield	Harvest index	Ethanol yield
Block	2	0.11	5.16	11174	0.45	8.23	171599
Planting date (D)	3	1.44**	26.3*	111148*	32.34**	325.03**	3717037**
Error a	6	0.04	1.83	7711	1.16	3.17	124111
Nitrogen level (N)	2	0.38**	5.13*	1221*	3.16	12.30	71932*
D × N	6	0.009	0.62	2569	1.83	3.82	15437
Error b	16	0.01	0.57	1549	1.46	4.01	144819
CV%		6.72	5.8	2.98	9.68	12.48	15.9

**, * significant at 1 and 5 percent level, respectively.

Table 9. Mean comparisons* for number of grain/panicle,1000 grain weight, grain yield, biological yield, harvest index and ethanol yield at four planting dates and three nitrogen levels.

Treatments	Number of grain/panicle	1000 grain weight/gr	Grain yield kg/ha	Biological yield kg/ha	Harvest index %	Ethanol yield l/ha
Planting dates						
July 5	1195.11^{d}	10.91^{c}	1437.73^{c}	14523.00^{a}	10.10^{c}	619.70^{c}
July 15	1298.22^{c}	13.03^{b}	1877.02^{b}	12879.00^{b}	14.56^{b}	809.00^{b}
July 25	1372.79^{b}	13.22^{b}	1997.80^{b}	12644.00^{b}	15.88^{b}	861.00^{b}
August 4	1457.57^{a}	15.07^{a}	2411.92^{a}	9945.00^{c}	24.40^{a}	1039.50^{a}
Nitrogen levels (kg/ha)						
50	1312.17^{b}	12.61^{b}	1805.21^{c}	12380.00^{a}	15.40^{a}	778.00^{b}
100	1312.33^{b}	12.77^{b}	1854.28^{b}	12050.00^{a}	15.88^{a}	779.20^{b}
150	1367.50^{a}	13.81^{a}	2133.87^{a}	13050.00^{a}	17.34^{a}	919.30^{a}

*Values within a column followed by same letter are not significantly different at 5% using Duncan Multiple Range Test.

July 5 and increased to 1039.50 l/ha in August 4 planting. Harvest index was 10.10 and 24.40 for July 5 and August 4 plantings, respectively. Biological decreased from 14523.00 kg/ha in July 5 planting to 9945.00 kg/ha in August planting. The effect of nitrogen levels on grain yield was significantly different at 1% level and for 1000

Table 10. Correlation coefficients for grain yield and its components.

Trait	Grain yield	1000 grain weight	Number of grain/panicle	Biological yield	Harvest index	Ethanol yield
Grain yield	1					
1000 grain weight	0.986**	1				
Number of grain in panicle	0.943**	0.888**	1			
Biological yield	-0.796**	-0.806**	-0.793**	1		
Harvest index	0.961**	0.961**	0.905**	-0.903**	1	
Ethanol yield	0.913**	0.926**	0.860**	-0.775**	0.925**	1

**Correlation is significant at the 0.01 level.

grain weight, number of grain/panicle and ethanol yield at 5% level. Mean comparisons for the aforementioned measurements are shown in Table 9. All the measurements were lowest with the application of 50 kg/ha urea and highest with the application of 150 kg/ha urea. Grain yield increased from 1805.21 kg/ha with the application of 50 kg/ha urea to 2133.87 kg/ha with the application of 150 kg/ha urea. 1000 g weight increased from 12.61 g to 13.81 g with the application of 50 and 150 kg/ha urea. Ethanol yield increased from 778.00 l/ha with application of 50 kg urea/ha to 919.00 kg/ha with the application of 150 kg urea/ha.

Correlation coefficient

Correlation coefficient for grain and its components are shown in Table 10. Grain yield is positively significantly correlated to 1000 grain weight, number of grain/panicle and harvest index, ethanol yield and negatively significantly correlated with biological yield. Grain yield, 1000 grain weight, number of grain/panicle and harvest index had significantly positive effect on ethanol yield.

Biological yield was significantly negatively correlated to grain yield, 1000 grain weight, number of grain/panicle and harvest index.

DISCUSSION

Stalk plant height, plant diameter, total fresh weight, and stem fresh weight

Significant decreases in plant height and plant diameter were found in sweet sorghum planted in August (Table 5). Plant height and plant diameter gradually decreased from the first planting date in July 5 to the last planting date in August. In general, long growing season increased plant height and plant diameter. Sweet sorghum plant height increased with earlier planting date (Rao et al., 2013; Almodares and Darany, 2006). July planting (July 5, 15 and 25) had similar and significantly more biomass (35393 kg/ha) than August planting (30780 kg/ha) (Table 5). Similarly, they had more stem fresh weight (30560 kg/ha) than August planting (21530 kg/ha). Earlier reports from temperate climates showed that sweet sorghum biomass and stalk yield decreased when planted late (June and July) rather than early planting (April and May) (Han et al., 2012; Teetor et al., 2011; Burks et al., 2013). Early plantings (May) had significantly higher fresh stalk yield than June planting (Almodares and Darany, 2006). Early planting allowed for a longer growing period and earlier canopy development for sunlight interception during the long days of June and July. Our results showed that planting sweet sorghum in early July was optimal for maximum stalk yield under this climatic condition.

In Khuzestan province where the experiment was conducted, growing sweet sorghum in May following wheat harvest was possible. Sweet sorghum is newly introduced to this area and farmers are used to planting corn at the end of July to reduce the risk of low pollination due to high temperature. If corn is planted early, farmers obtain low yield due to coincidence of hot temperature with pollination. Following wheat harvest, agricultural lands are in fallow for nearly two months before corn planting. When considering the introduction of sweet sorghum, the acceptance of farmers and their willingness to integrate ecologically appropriate crops must be guaranteed. The motivation of farmers may increase if sorghum cultivars provide direct benefits, such as acceptable biomass and biofuel production from land where other crops are unproductive (Almodares and Hadi, 2009). Planting sweet sorghum early in the season will produce the highest biomass due to long growing season before panicle initiation. Sorghum is a short day plant, with panicle initiation starting when day length reaches 12.0 h (Miller et al., 1968). This suggests that planting sweet sorghum can be staggered from July 5 to July 25 without appreciate reduction of biomass (Table 5). Biomass in August planting was reduced considerably because days from planting to panicle initiation was not long enough for vegetation phase. Higher total biomass and stalk yield of sweet sorghum from early planting may be due to higher total accumulated thermal time and higher ambient air and soil temperatures associated with

longer day length during pre-flowering stages (Rao et al., 2013).

Physiological mechanisms in plants are capable of sensing differences in day length. Under most conditions, late planting is associated with a reduction in number of days to panicle initiation and flowering, which affect temperature and photoperiod (Balole, 2001). For the first planting dates, the growing season from planting to flowering was long and assimilates were used for vegetative growth. For the last planting date the growing season was short and there was not much time for vegetative growth. Temperature stresses (high and low temperature) are the major environmental factors affecting plant growth and development. They also induce morphological, physiological and biochemical changes in plants (Waraich et al., 2012). High temperature decreases photosynthesis (Wise et al., 2004) which causes shoot and root growth inhibition and yield reduction in plants (Vollenweider and Gunthardt-Goerg, 2005). Due to the high temperature in August planting, stem height, stem diameter, total fresh weight and stem fresh weight were significantly lower than the other planting dates. High performance of sweet sorghum in early planting could be due to long growing season which coincide with more fresh leaves, higher photosynthesis (Net Assimilation Rate, NAR), and more growth (relative growth rate, RGR and crop growth rate, CGR). Although stem height, stem diameter, total fresh weight and stem fresh weight were higher with the application of 150 kg/ha urea than other nitrogen applications, but their differences were not significant (Table 5). Higher N application of 150 kg/ha recorded higher sweet sorghum biomass compared to lower dose of N application (Poornima et al., 2008). Erickson et al. (2011) reported that biomass was not affected by N fertilization rate. It seems high rate of nitrogen was not uptake by reduced roots due to high temperature and consequently lower plant growth and development.

Brix, total sugar, sucrose, purity and ethanol yield

Early plantings (July 5 and July 15) had higher brix, total sugar, sucrose and purity than late plantings (July 25 and August 4 (Table 6). Sweet sorghum produced higher sugar and ethanol yields in early plantings (April to May) in temperate (Han et al., 2012; Teetoor et al., 2011) and Mediterranean climates in Egypt (El-Razek and Besheit, 2009). Delaying planting from mid-June to early July and mid-July reduced the average estimated total ethanol yields for six cultivars from 5189 to 3852 and 2808 L ha^{-1}, respectively (Houx and Fritschi, 2013). Sweet sorghum was planted in May 4, March 19, June 4 and June 18 (Almodares and Darany, 2006). Results showed plant height, plant diameter, fresh stalk yield, total dry weight, brix value, sugar content and grain yield was higher in the first planting date than the other planting dates. Planting of full season varieties commonly increases potential ethanol yield (Zhao et al., 2009; Putman et al., 1991). The results of monthly planting of sweet sorghum in a temperate climate showed May plantings produced more fermentable sugars, sugar, and ethanol yields than other months (Han et al., 2012; Teetor et al., 2011). In published papers, the effect of nitrogen fertilizer on sweet sorghum brix value is contradictory. Nitrogen application did not increase fresh stalk yield, brix value and sugar yield and the mean brix value was negatively related to N rate (Almodares and Darany, 2006). On the other hand, both stalk and juice yield increased with increasing rates of nitrogen application (Kumar et al., 2008). Although sweet sorghum responds well to fertilizers, especially nitrogen but our results showed (Table 5 and 7) showed that sweet sorghum performance was not affected by application of nitrogen fertilizer. It seems that the performance of sweet sorghum with the application N application varies under different environmental conditions.

Grain and its components

Number of grain/panicle, 1000 grain weight, grain yield, biological yield, harvest index and ethanol yield were affected by planting dates (Table 8). The aforementioned measurements were significantly highest in the last planting date (August 4) and lowest in the first planting date (July 5). In contrast, biological yield was highest in the first planting date and lowest in the last planting date. Planting dates significantly affected biomass. Harvest index is related to number of grain per panicle, 1000 grain weight and grain yield. Harvest index increased from July 5 and it was highest in August 4. Grain yield reduction in July could be due to hot temperature and dry weather condition which reduces flowering. Thus farmers in the experiment site area plant corn in August, so the plant pollinates when the temperature is cool enough not to reduce pollination. The climatic condition in tropics ismuch different from temperate. Almodares and Darany (2006) reported sweet sorghum fresh stalk weight, sugar yield, grain yield and its components were highest in May planting and lowest in June planting. The results showed grain yield was significantly increased by nitrogen application. It was highest with application of 150 kg urea/hectare. This could be due to the number of grain per panicle and 1000 grain weight which were higher with the application of 150 kg urea per hectare than other nitrogen applications. Our results are in agreement with (Kaufman et al., 2013; Miko and Manga, 2008). Ethanol yield was highest with the application of 150 kg urea/ha and lowest with the application of 50 kg urea/ha. Ethanol yield is positively correlated with grain yield (Table 10).

Correlation coefficients

Both biomass and carbohydrate content had effect on

ethanol yield, therefore ethanol yield will be increased by higher growth parameters such as stem height, stem diameter, total fresh weight, etc and carbohydrate content: brix value, sucrose content and purity. Ethanol yield was significantly positively correlated with stem height, stem diameter, stem fresh weight and brix value, sucrose content and purity (Table 8) and grain yield, 1000 grain weight and of number of grain/panicle (Table 10). It significantly negatively correlated with biological yield. Biological yield refers to the total dry matter accumulation of a plant system. If more photosynthates translocate to vegetative organs, such as stem and leaves, there is less photosyntates to translocate into organs having economic yield such as grain and vice versa. So, biological yield and grain have negative correlation.

Total ethanol yield

Stem ethanol yield was highest in the first planting date (July 5) and lowest in last planting date (August 4). On the other hand, grain ethanol yield was highest and lowest in last planting date and first planting dates, respectively. Stem ethanol yield significantly correlated to plant growth and developments and carbohydrates (Table 7). All of these characteristics will be reduced by late planting. Grain yield and its components increased with late planting because flowering coincide with cool temperature. Grain ethanol yield is significantly correlated with grain and its components (Table 10). Since ethanol production from stem is several times more than ethanol from grain, therefore total ethanol from stem and grain was higher in the first and second planting dates 3484 and 3431 l/ha, respectively than third planting date (3250 l/ha). Lowest total ethanol yield was obtained (2514 l/ha) in last planting date.

Conflict of Interests

The authors have not declared any conflict of interests.

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Silicon and excess ammonium and nitrate in cucumber plants

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Excess ammonium and nitrate are associated with physiological disorders in plants; however, these disturbances can be minimized with the use of silicon, especially in plants supplied with ammonium. The objective of this study was to evaluate the effect of silicon on the presence of excess ammonium and nitrate in two cucumber varieties (*Cucumis sativus*) on physiology and growth of the plants. The experiment was carried out in hydroponic cultivated cucumber plants, at the São Paulo State University, Brazil. A completely randomized design was used with four replications, in a 2 × 3 × 2 factorial corresponding to two sources of nitrogen (ammonium and nitrate) at a concentration of 10 mmol L^{-1}, three silicon concentrations (0, 1 and 10 mmol L^{-1}) and two varieties of cucumber (Tsubasa and Hokushin). At 28 days after treatment application, evaluations were performed for silicon and nitrogen accumulation in the shoots, green color index, number of stomata, nitrate reductase activity, height, leaf number and dry matter mass. Silicon promoted an increase in the growth variables and improved the physiological parameters of the plants only when supplying the ammonium N source. The use of Si, independent of the cucumber variety, mitigated the toxicity of ammonium, resulting in greater total nitrogen accumulation and dry matter of plants; however, it did not benefit the plants under excess nitrate nitrogen.

Key words: *Cucumis sativus*, nitrogen, nutritional disorder, beneficial element, stress.

INTRODUCTION

Nitrogen (N) can be absorbed by plants in both the ammonium and nitrate forms, and these absorption forms can affect many physiological and biochemical processes including photosynthesis, enzymatic activities of the N metabolism (Lasa et al., 2002; Cruz et al., 2011), development and morphology of the plant (Zhu et al., 2000).

Ammonium excess causes toxicity in plants, changes in

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intracellular pH, damage to the osmotic balance, metabolism of plant hormones and polyamines, and also induces mineral nutrient deficiency (Gerendas et al., 1997). Summed with these factors, excess ammonium nitrogen also promotes an increase in the content of O_2^- and H_2O_2, inducing oxidative stress, lower production of chlorophyll and carotenoids (Wang et al., 2010), and consequently lower photosynthetic rates (Su et al., 2012), resulting in reduced growth, chlorosis and necrosis of leaves and roots (Wong, 2005).

Nitrogen, when absorbed by plants in the nitrate form, even in excess, does not cause damage to plants because most species have a mechanism for nitrate accumulation in the vacuoles (Silveira and Crocomo, 1990), resulting in no signs of toxicity (Lasa et al., 2001, 2002). However, although its excessive accumulation does not harm the plant, consumption of these plants can cause harm to humans and animals (Santamaria, 2006).

Plant species have different tolerances to excess N (Horchani et al., 2011). Some plants are more sensitive to excessive N (ammonium and nitrate), such as *C. sativus* (Roosta et al., 2009), especially ammonium, presenting toxicity symptoms at concentrations above 5 mmol L^{-1} ammonium, causing reduced growth and development of plants (Roosta and Schjoerring, 2008; Roosta et al., 2009) and losses in dry matter accumulation (Roosta and Schjoerring, 2007). However, varieties of the same species may present differences regarding tolerance of excess N (Cruz et al., 2011), which could also vary in function of the form of N provided.

To relieve stresses in plants, one option is the selection and the use of genotypes that are tolerant to certain stress. Another alternative is the use of beneficial elements, such as silicon (Si), which can reduce or alleviate stress caused by excess N in plants (Ma and Yamaji, 2006). Therefore, plants that accumulate Si, for example *C. sativus* (Liang et al., 2005) potentially have greater capacity to alleviate stress caused by absorption of high concentrations of N.

Si is important to mitigate abiotic stresses caused by nutritional imbalances in plants (Ali et al., 2013). Excess N causes lodging and shading of the plants and with the use of Si these effects can be reduced due to deposition of silica in stems and leaf blades (Ma, 2004). Furthermore, the effect of Si on stress mitigation can be attributed to its involvement in metabolic and physiological activities of plants (Shen et al., 2010).

Si promotes alterations in the anatomy of leaves, increasing thickness, leaf area (Farshidi et al., 2012), stomatal conductance (Ali et al., 2013; Shi et al., 2013) and promoting photosynthetic mechanisms (Mateos-Naranjo et al., 2013) in plants under stress conditions.

Associated with this, genotypes with more stomata may promote increases in photosynthesis and consequently the synthesis of carbon skeletons, culminating in increased assimilation of ammonia N and decreasing toxicity due to excess of this ion (Roosta and Schjoerring, 2008).

The beneficial effects of Si may be related to increased antioxidant activity, reducing lipid peroxidation of the leaves (Jiao-Jing et al., 2009) and protecting and helping to maintain them. This may be reflected in the chlorophyll and green color index of plant leaves.

Studies involving Si and excess ammonium and nitrate are still incipient in literature, generated a need to increase research in this area. Therefore, the hypothesis suggested is the use of Si to mitigate the effects of excess N in its ammonium and nitrate forms, independent of the cucumber variety cultivated.

The objective of the present study was therefore to evaluate the effect of silicon on the presence of excess ammonium and nitrate in two cucumber varieties (*C. sativus*) with regards to physiology and growth of plants.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at the Department of Soils and Fertilizers, FCAV/UNESP – Jaboticabal Campus, SP, with geographic coordinates of 21° 15' 22'' South, 48° 18' 58'' West and elevation of 575 m, with cucumber plants (*Cucumis sativus*) grown hydroponically for 43 days.

The experiment was setup in a completely randomized design, with four replications. The treatments were arranged in a 2 × 3 × 2 factorial, corresponding to two nitrogen sources (ammonium and nitrate), applied at a concentration of 10 mmol L^{-1}, three silicon concentrations (0, 1 and 10 mmol L^{-1}) and two cucumber varieties (Hokushin and Tsubasa). Ammonium chloride and calcium nitrate were used as sources of ammonium and nitrate N, respectively, and potassium silicate as the source of Si. Concentrations of potassium and calcium were balanced between treatments so that they were maintained uniform, the sources were potassium chloride and calcium chloride, respectively.

Each experimental unit consisted of a polypropylene pot with a lid, measuring 48 cm long, 11 cm wide at the lower base, 16 cm wide at the upper base and 17 cm tall, containing 8 L of nutrient solution and six cucumber plants.

Seeds were sown in styrofoam trays with 288 cells, in substrate consisting of ground coconut husk. The plants remained in a greenhouse with controlled humidity conditions until presenting three leaves (15 days after germination), and after this period were transplanted to polypropylene pots. To fixate the plants in the holes present on the pot lids a cooler was used, and a string for staking each plant. From then on, the plants were grown in nutrient solution, proposed by Hoagland and Arnon (1950), using the N concentration according to the treatments. The nutrient solution was maintained under continuous oxygenation by means of an air compression system.

The nutrient solution was exchanged weekly and the treatments were added in stages to prevent early toxicity due to excess nitrogen and impair the effect of silicon on the plants. Therefore, in the first week the complete nutrient solution with 50% ionic strength was used, but that the nitrogen concentration was 25% and silicon concentrations were 50%. As of the second week of cultivation the nutrient solution of Hoagland and Arnon (1950) was used with 100% ionic strength, 50% nitrogen concentrations and 100% silicon concentrations. On the third week the nitrogen concentration was increased to 100% and silicon concentration remained at 100% in the nutrient solution.

The nutrient solution used was that proposed by Hoagland and Arnon (1950), modified by changing the iron source to Fe-EDDHMA.

Water used in the hydroponic system was distilled and deionized, where solution levels were completed daily in each vessel with stock solutions corresponding to each treatment. Values of pH were adjusted to between 5.5 and 6.0 using solutions of HCl 1.0 mol L^{-1} or NaOH 1.0 mol L^{-1}.

After 28 days of treatment application, four plants were selected per experimental unit and the following variables were assessed: height, leaf area, green color index, the nitrate reductase enzyme activity, shoot dry matter mass and a single plant was selected for counting the number of stomata.

Measurements of height were made from the base of the styrofoam to the apical meristem of the main stem with the aid of ruler graduated in centimeters. The leaf area measurements were performed by collecting all the leaves per plant followed by determination using an integrator portable area meter (LI-Cor® LI-3000C model). The green color index was determined on the third developed leaf from the apex, in the middle third of the leaves, during the period between 11:00 and 12:00 h in the morning, obtained with the aid of a green color index meter (Opti-Sciences® CCM-200 Chlorophyll Meter).

The number of stomata was measured on the third developed leaf from the apex between 07:00 and 08:00 h in the morning, followed by preparation of three slides per leaf. Thus, a cyanoacrylate-based adhesive was applied to the median portion of the abaxial leaf surface followed by securing the leaf on the glass slide for two minutes, obtaining impression of the epidermal surface on the slide. For display, we used microscopy with 40x objective lens. On each slide the number of stomata in three fields of vision was counted, where each field corresponded to 0.1 mm^2 of the leaf surface.

Activity of the enzyme nitrate reductase was measured in the fourth developed leaf from the apex of the plants with the nitrate N source. For this the leaves were collected between 10:00 and 11:00 h in the morning and soon after the analysis was performed in triplicate with the leaves *in vivo* according to the methodology proposed by (Cazetta and Villela, 2004).

To obtain the dry mass of the shoots, the plants were collected, the shoots separated, washed, packaged in paper bags, dried in a forced air circulation oven (65°C) until constant weight and after drying the material was weighed on a precision scale. After obtaining the dry mass of the shoots, the material was ground in a Wiley mill and nitrogen concentrations were determined according to the methodology proposed by Bataglia et al. (1983) and silicon according to Kraska and Breitenbech (2010). From the nitrogen and silicon contents and the accumulation of dry shoot mass, the accumulation of these elements in the plant was calculated.

The obtained data was submitted to analysis of variance by the F-test, followed by application of the Tukey test at 5% probability for comparison of the means, using the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

Accumulation of N and Si

Nitrogen accumulation in cucumber plants was influenced by the effect of the interaction among varieties and nitrogen sources and the interaction between nitrogen sources and silicon concentrations in the nutrient solution (Table 1).

Upon comparison of the two varieties, when nitrogen was supplied via the ammonium source, greater accumulation of the nutrient was observed in the Tsubasa variety. However, when N was supplied in the nitrate form, the Hokushin variety accumulated more N in the shoots (Table 2). Considering only the nitrogen sources, it was found that regardless of the cultivar, when provided as a nitrate source there was greater accumulation of N in the shoots of cucumber plants. Similar results were reported by Cruz et al. (2011) when studying four varieties of pea (*Pisum sativum* L) subjected to sources of N (ammonium and nitrate), finding that the varieties have different adaptive responses to excess ammonium. This fact may be attributed to distinguishing features already existent among plant species (Lasa et al., 2002) and even varieties (Cruz et al., 2011) with regards to nitrogen fertilization.

The accumulation of nitrogen in plants, independent of N supplied from ammonium or nitrate sources, was greater in the presence of Si with no difference between the concentrations of 1 and 10 mmol L^{-1} (Table 2). When evaluating the effect of N concentrations (nitrate and ammonium) and addition of Si in *Brassica napus* L. plants, Bybordi (2010) observed a beneficial ratio of Si with excess NH_4^+ to promote increases in production of fresh weight and leaf area. According to Ma and Yamaji (2006), the stress caused by high N doses can be reduced or mitigated by use of Si.

The relationship of Si with N has also been verified by Bybordi (2012) and Feng et al. (2010) when reporting that in the presence of this beneficial element there was increased activity of enzymes acting in the N metabolism, such as nitrate reductase, glutamine synthetase, glutamate synthetase and glutamate dehydrogenase. This beneficial effect may be even more significant in Si accumulating plants, such as *C. sativus* (Liang et al., 2005).

Silicon accumulation in cucumber plants was influenced by the following interactions: Varieties and nitrogen sources, varieties and Si concentrations, and N sources and Si concentrations (Table 1). Accumulation of this beneficial element in shoots of the varieties studied was higher in plants nourished with N in its nitrate form. When submitted to this nitrogen source increases of 111.8 and 65.9% in Si accumulation were observed in the Hokushin and Tsubasa varieties, respectively (Table 2). For the nitrate source, there was no difference in Si accumulation between varieties, however for the ammonium source the Tsubasa variety presented greater accumulation (Table 2).

In the absence of Si, for both N sources the plants presented lower accumulation of the element in relation to the treatment with silicon. No effect was observed on the accumulation of Si among the concentrations of 1 and 10 mmol L^{-1} of the beneficial element, independent of the variety and N source (Table 2).

Both cucumber varieties showed higher N and Si accumulation when submitted to the nitrate source compared to the ammonium source. This result is attributed to the fact that excess nitrate does not cause toxicity to plants (Lasa et al., 2001, 2002). However,

Table 1. Accumulation of nitrogen (N), silicon (Si) and the green color index (GCI) of two varieties of *Cucumis sativus* in function of silicon and nitrogen application.

Varieties (V)	Nutrient accumulation (mg plant^{-1})		
	N	Si	GCI
Hokushin	137.82	6.33	27.24
Tsubasa	145.47	6.71	30.67
MSD	4.71	0.27	0.89
Si (Si)			
0 mmol L^{-1}	110.76	3.08	25.67
1 mmol L^{-1}	157.04	8.20	30.62
10 mmol L^{-1}	157.13	8.29	30.58
MSD	6.95	0.40	1.31
Nitrogen (N)			
NO_3^-	174.26	8.49	31.63
NH_4^+	109.03	4.56	26.28
MSD	4.71	0.27	0.89
		F-test	
V	10.86**	8.06**	61.81**
Si	177.23**	653.61**	56.58**
N	790.77**	853.37**	149.73**
V x N	111.49**	19.90**	25.61**
V x Si	0.07^{ns}	3.59*	0.04^{ns}
N x Si	75.61**	17.62**	36.96**
V x N x Si	0.12^{ns}	2.00^{ns}	1.78^{ns}
CV (%)	5.7	7.2	5.2

*, ** and ^{ns}: Significant (P<0.05), (P<0.01) and non-significant by the F-test, respectively. MSD: minimum significant difference.

Table 2. Effects of the interactions between varieties, silicon and nitrogen concentrations for accumulation of nitrogen, silicon and the green color index (GCI) of two varieties of the *Cucumis sativus* plant.

Varieties (V)	Nitrogen sources (N)					
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	N (mg plant^{-1})		Si (mg plant^{-1})		GCI	
Hokushin	92.95^{bB}	182.69^{aA}	4.06^{bB}	8.60^{aA}	23.45^{bB}	31.02^{aA}
Tsubasa	125.09^{aB}	165.83^{bA}	5.05^{aB}	8.38^{aA}	29.10a^{B}	32.24^{aA}
MSD (V)	6.65		0.39		1.25	
MSD (N)	6.65		0.39		1.25	
Silicon (Si)						
0 mmol L^{-1}	57.99^{bB}	163.53^{bA}	1.67^{bB}	4.49^{bA}	20.34^{bB}	30.99^{aA}
1 mmol L^{-1}	135.33^{aB}	178.75^{aA}	6.02^{aB}	10.38^{aA}	29.48^{aB}	31.76^{aA}
10 mmol L^{-1}	133.75^{aB}	180.51^{aA}	5.98^{aB}	10.61^{aA}	29.01^{aB}	32.14^{aA}
MSD (Si)	9.83		0.57		1.85	
MSD (N)	8.15		0.47		1.54	

Equal lower-case letters in the columns do not differ by the Tukey test (P<0.05). Equal upper-case letters on the lines do not differ by the Tukey test (P<0.05). MSD: minimum significant difference.

excess ammonium N generates damage to the root system, causing necrosis and reducing growth (Wong, 2005), further resulting in lower absorption of water, nutrients and Si, and therefore reduced plant growth.

Green color index

The green color index (GCI) was influenced by the interactions between varieties and N sources, and N sources and silicon concentrations (Table 1). In both varieties the GCI was higher in treatments where N was provided in the nitrate form. These results can be attributed to excess ammonium causing a decrease in chlorophyll levels (Wang et al., 2010) with consequent chlorosis in the leaves (Wong, 2005) and increased synthesis of putrescine, evolving to necrosis of leaf tissues (Prado, 2008), resulting in the lower green color index found in this study.

Upon comparing the two varieties, when fertilized with nitrate N no differences were observed in the GCI, however when fertilized with ammonium N it was found that Tsubasa presented a higher GCI in relation to Hokushin (Table 2).

The use of Si promoted an increase in the GCI of plants fertilized with ammonium N (Table 2). However, there was no difference between the concentrations of 1 and 10 mmol L^{-1} of the beneficial element, due to the absence of differences in Si accumulation between these concentrations (Table 2). It is known that Si promotes protective effects to photosynthetic mechanisms, and the balance of nutrients (Mateos-Naranjo et al., 2013), increasing the antioxidant activity of plants and decreasing lipid peroxidation of the leaves (Jiao-Jing et al., 2009). These benefits to the plant metabolism contribute to maintain the leaves photosynthetically active, thus mitigating the harmful effects of ammonium toxicity to the plants, resulting in an increase in chlorophyll synthesis that is reflected in a greater GCI.

Although silicon promoted an increase in the green color index in plants cultivated with excess ammonium N, in plants cultivated with excess nitrate N the use of this beneficial element had no effect on the same variable (Table 2). The use of Si only benefited the plants submitted to excess ammonia, and is explained because excess nitrate does not cause toxicity to *C. sativus* plants, where toxicity is caused only by the use of ammonium (Roosta and Schjoerring, 2007), thus indicating the benefits of Si on plants under stress conditions.

Enzymatic activity and number of stomata

Activity of the nitrate reductase enzyme presented a significant effect ($P<0.01$) for the cucumber varieties, with higher values in the variety Hokushin than in Tsubasa. No significant effects were evident for Si concentrations or for interaction between varieties and Si concentrations (Table 3). These results are similar to those found by Bybordi (2010) in studies involving Si and N in *Brassica napus* L. plants.

The number of stomata was influenced only by the varieties, where the variety Tsubasa has a greater number of stomata when compared to the variety Hokushin. Although changes are reported in stomatal conductance with the use of high ammonium concentrations (Lopes and Araus, 2006), there was no effect between N sources and interactions (Table 3).

Despite the fact that Si promoted changes in the anatomy of leaves, increasing the thickness, leaf area (Farshidi et al., 2012) and stomatal conductance in plants under stress conditions (Ali et al., 2013; Shi et al., 2013), no effect of the Si concentrations and interactions was observed regarding the number of stomata.

Growth and production of dry matter mass

The growth of cucumber plants, evaluated by the height, leaf area and dry matter accumulation, was influenced by the interaction between varieties and nitrogen sources and the interaction of nitrogen sources with silicon concentrations (Table 3).

Plant growth evaluated with regards to height, leaf area and accumulated of dry matter mass, when fertilized with ammonium N, was always higher in the variety Tsubasa, however when the N source was nitrate, the highest values for all of these variables were obtained from plants of the variety Hokushin (Table 4).

These results can be attributed to the different responses to excess N by the plant varieties (Cruz et al., 2011) due to the characteristics of each species and variety regarding the site of organic ammonium N incorporation (Lasa et al., 2002), and enzyme activities that participate in the detoxification process of excess N (Doubnerová and Ryslavá, 2011).

There was greater growth of plants under the nitrate N source, promoting increases of 65.3 and 19.2% for height, 141.9 and 82.8% for leaf area, and 136.4 and 63.1% for the accumulated mass of dry matter in the varieties Hokushin and Tsubasa, respectively (Table 4).

This occurred because the excess ammonium N caused toxicity in *C. sativus* plants, which are characterized as sensitive to excess ammonium (Roosta et al., 2009). Similar results were reported by Roosta and Schjoerring (2007) in studies with *C. sativus* plants and N sources (nitrate and ammonium), confirming that the accumulated mass of dry matter was lower in plants cultivated with ammonium N in relation to those cultivated with nitrate N.

Excess ammonium in *C. sativus* plants causes imbalances between nutrients, leading to deficiency of calcium and magnesium (Roosta and Schjoerring, 2007), with harmful consequences to plant nutrition, and therefore growth and production of dry matter. The ammonium concentration of 10 mmol L^{-1} applied to the nutrient solution is considered high and toxic to the plants tested in this study. According to Roosta and Schjoerring (2008) and Roosta et al. (2009), ammonium concentrations

Table 3. Activity of nitrate reductase, number of stomata (NS), height, leaf area and accumulation of dry matter mass (DM) for plants of two varieties of *C. sativus*, in function of the application of silicon and nitrogen.

Varieties (V)	Nitrate reductase (μmol g^{-1} h^{-1})	NS	Height (cm)	Leaf area (cm^2)	DM (g/plant)
Hokushin	42.20[a]	44.92[b]	88.13	989.58	3.28
Tsubasa	36.88[b]	49.08[a]	91.04	1004.21	3.35
MSD	1.52	3.80	1.84	46.88	0.09
Si (Si)					
0 mmol L^{-1}	39.69	47.12	76.44	868.38	2.85
1 mmol L^{-1}	39.58	46.75	96.00	1056.77	3.54
10 mmol L^{-1}	39.36	47.13	96.31	1065.53	3.56
MSD	2.26	5.62	2.72	69.23	0.14
Nitrogen (N)					
NO_3^-	-	46.50	104.42	1349.20	4.38
NH_4^+	-	47.50	74.75	644.59	2.25
MSD	-	3.80	1.84	46.88	0.09
			F-test		
V	53.90**	4.93*	10.35**	0.40[ns]	2.67[ns]
Si	0.07[ns]	0.02[ns]	210.34**	30.96**	104.84**
N	-	0.28[ns]	1071.01**	929.13**	2200.74**
V x N	-	0.39[ns]	230.07**	25.51**	130.21**
V x Si	0.04[ns]	0.16[ns]	0.65[ns]	1.27[ns]	0.90[ns]
N x Si	-	0.73[ns]	189.55**	27.88**	86.71**
V x N x Si	-	1.33[ns]	0.46[ns]	1.39[ns]	2.82[ns]
CV (%)	4.5	13.8	3.5	8.0	4.8

*, ** and [ns]: Significant (P<0.05), (P<0.01) and non-significant by the F-test, respectively. MSD: Minimum significant difference.

Table 4. Effects of the interactions between varieties, silicon and nitrogen for height, leaf area and accumulation of dry matter mass (DM) for two varieties of *C. sativus* plants.

Varieties (V)	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
	Height (cm)		Leaf area (cm^2)		DM (g $plant^{-1}$)	
Hokushin	66.42[bB]	109.83[aA]	578.90[bB]	1400.26[aA]	1.95bB	4.61[aA]
Tsubasa	83.08[aB]	99.00[bA]	710.28[aB]	1298.14b[A]	2.55[a]B	4.16b[A]
MSD (V)	2.60		66.30		0.13	
MSD (N)	2.60		66.30		0.13	
Silicon (Si)						
0 mmol L^{-1}	49.13[bB]	103.75[aA]	394.07[bB]	1342.69[aA]	1.36bB	4.34[aA]
1 mmol L^{-1}	87.25[aB]	104.75[aA]	768.20[aB]	1345.35[aA]	2.70[a]B	4.37[aA]
10 mmol L^{-1}	87.88[aB]	104.74[aA]	771.51[aB]	1359.55[aA]	2.69[a]B	4.44[aA]
MSD (Si)	3.84		97.90		0.19	
MSD (N)	3.18		81.20		0.16	

Equal lower-case letters in the columns do not differ by the Tukey test (P<0.05). Equal upper-case letters on the lines do not differ by the Tukey test (P<0.05). MSD: minimum significant difference.

above 5 mmol L^{-1} cause damage to the growth and development of *C. sativus* plants.

The low tolerance to excess ammonium by *C. sativus* plants may be associated with the location of ammonium

assimilation, where most is assimilated in shoots (Roosta et al., 2009) and also by enzyme activity (glutamate dehydrogenase and glutamine synthetase) (Cruz et al., 2006). However, excess nitrate is tolerable by most plant species, because it is accumulated in vacuoles (Lasa et al., 2001, 2002), thus showing no symptoms of toxicity.

The use of Si in the nutrient solution at concentrations of 1 and 10 mmol L^{-1} promoted the best results for the variables of height, diameter, leaf area and accumulation of dry matter mass in plants supplied with ammonium N, however response of the beneficial element on plants supplied with nitrate N was not verified (Table 4). The beneficial effect of silicon on the growth of *C. sativus* plants subjected to stress conditions was confirmed by Zhu et al. (2004) when establishing that the use of Si (1 mmol L^{-1}) promoted an increase in the growth variables of plants under salt stress, resulting in higher accumulation of dry matter mass. These results, attributed to the fact that Si reduced the oxidative damage of membranes caused by salinity, indicated greater plant growth.

The Si promoted increased antioxidant activity in plants, reducing lipid peroxidation, providing protection to plants against oxidative damage, and increased plant growth and development (Jiao-Jing et al., 2009). These effects on growth induced by Si in conditions of excess ammonium may be associated with its protective effects on the photosynthetic apparatus of plants (Mateos-Naranjo et al., 2013) because excess ammonium has harmful effects on photosynthesis (Britto and Kronzucker, 2002), such as lowering of the green color index (Table 1). Thus the use of Si possibly mitigated this effect of ammonium toxicity.

Although silicon promoted growth of the plants when supplied with ammonium N, no difference was observed between the concentrations of 1 and 10 mmol L^{-1} of this beneficial element, indicating that there were no losses in plant development when cultivated with high concentrations of Si. It was noted that this fact may possibly be explained by the occurrence of Si polymerization in the nutrient solution with increase in its concentration, reducing its absorption. Another explanation is that most of the absorbed Si, about 90%, is immobilized as biogenic opal on the outside of the cell wall (Silva and Bohnen, 2001), with only a small free amount in the plant.

Conclusion

The use of Si independent of the *C. sativus* variety mitigates the toxicity of ammonium, resulting in higher accumulations of total nitrogen and dry matter of plants; however, it does not benefit plants under excess nitrate N.

Conflict of Interests

The authors have not declared any conflict of interest.

Abbreviations

MSD, minimum significant difference; **EDDHMA**, ethylenediamine-N, **N-bis 2**, hydroxy-methyl phenylacetic acid; **GCI**, green color index; **DM**, dry matter mass; **NS**, number of stomata.

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Ecophysiological and biochemical behavior in young plants of *Parkia gigantocarpa* Ducke subjected to waterlogging conditions

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The *Parkia gigantocarpa* is a neotropical tree that naturally occurs in terra firme forest and floodplain. The aim of the study was to evaluate the physiological and biochemical behavior in young plants of *P. gigantocarpa* subjected to waterlogging conditions. The waterlogging was imposed at approximately 5 cm above the blade surface of the soil using pots with a capacity of 14 kg of substrate. The experimental design was completely randomized with two water conditions (control and waterlogging) combined with five evaluation times (0, 4, 8, 12 and 16-days waterlogging conditions). The variables evaluated were: Predawn water potential ($_p\Psi$); the foliar xylem water potential ($_x\Psi$); hydraulic conductivity; concentration of nitrate; nitrate-reductase activity (NRA); glutamine-synthetase (GS); total soluble amino acids; proline; glycine-betaine; alcohol-dehydrogenase (ADH) and lactate-dehydrogenase activity (LDH). The significant reduction in $_x\Psi$ and hydraulic conductivity continued until the 8th day in plants subjected to waterlogging, with subsequent stabilization. The concentration of nitrate, NRA, GS, and total soluble amino acids reduced significantly, in the leaves of plants subjected to waterlogging. The waterlogging increased the proline and glycine-betaine, mainly, in the leaves. The ADH activity was significantly higher in the root of the flooded plants, especially, on the 16th day of flooding. In the same period, the LDH activity showed the highest values, mainly, in the leaves of flooded plants. The results showed susceptibility of young plants of *P. gigantocarpa* subject to waterlogging conditions.

Key words: Water potential, proline, glycine-betaine.

INTRODUCTION

The waterlogging conditions has become a concern for the survival of plants in many regions of the world, being considered one of the main limiting abiotic factors of growth, the distribution of plant species, the crop yield

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(Jackson and Colmer, 2005) and forest plantations. The low diversity of tree species that can be used for recovery of areas in waterlogging conditions is a major challenge for environmental managers, because of the difficulty of species with adaptations to tolerate conditions of absence of oxygen (Lopez and Kursar, 2003). Thus, the identification of species that tolerate the waterlogging, including tree species with economic and environmental potential, is a viable alternative for the recovery of degraded areas.

An alternative is the cultivation of *Parkia gigantocarpa*, characterized as a neotropical tree that naturally occurs in terra firme forest and waterlogging conditions. Due to present rapid growth, uniformity, low death rate, economic and ecological potential, the tree is indicated as promising species for recovery of altered or degraded areas, especially those of permanent preservation, too, can be used in forestry systems. The waterlogging condition promotes the absence of oxygen in the soil, reducing the root system and the vegetation growth of trees which negatively affects the physiological processes of plants, including stomatal conductance, photosynthesis, and the hydraulic conductivity of the root and the synthesis and translocation of assimilates (Parent et al., 2008).

The stomatal closure contributes to the preservation of leaf water potential, and decreasing the reduction of hydraulic conductivity of roots (Kreuzwieser et al., 2004). Thus, the stomatal closure, the reducing evaporation, and the maintenance of leaf water potential are strategies developed by plants in waterlogged conditions to minimize water loss (Davanso et al., 2002). The root system is the respiratory glucose dissimilation of glucose fermentation with low output power (Drew, 1997), resulting in decreased absorption of nutrients for the plants (Rocha et al., 2010), interfering with the absorption of nitrate, due to change in nitrate-reductase activity, thereby influencing the nitrogen metabolism, amino acids (Liao and Lin, 2001), proteins and enzymes (Sairam et al., 2008).

Alam et al. (2011) observed that plants subjected to waterlogging conditions showed sugar decomposition processes, such as glycolysis and fermentation, associated with induction of synthesis of various stress proteins. The enzymes of fermentation pathways as lactate dehydrogenase, pyruvate decarboxylase, and alcohol-dehydrogenase are synthesized under conditions of absence of oxygen (Zabalza et al., 2009), thus, the activity of these enzymes are indicative of a possible adaptation of the plant response to waterlogging conditions, although it was not explained the extent of the contribution and how they relate to low-O_2 (Dennis et al., 2000).

Recommendation of *P. gigantocarpa* by farmers is necessary to obtain information about their ecophysiological and biochemical behavior in waterlogged conditions. The understanding about the behavior of tree species in the early stages of its development under waterlogging conditions can support reforestation of degraded areas. In this sense, the objective of the study was to evaluate the ecophysiological and biochemical behavior of young plants of *P. gigantocarpa* subjected to waterlogging conditions.

MATERIALS AND METHODS

Plant materials

The *P. gigantocarpa* (Ducke) seeds were collected from location 03°41' 07" S and 48°38' 04" W. Seeds were scarified in the lateral region and adjacent to the hilum, and were immediately sown in plastic trays with capacity of 5 L, containing sterile sand. After emergence, the seedlings were transplanted to black opaque polythene bags with dimensions of 25 x 15 cm in height and diameter, respectively, with perforations in all sides, containing the substrate Plantmax® (composed of composted pine bark, peat, vegetal charcoal, and vermiculite). The seedlings were grown for 60 days. After this period, the young plants were evaluated for height, stem diameter, number of leaves and number of leaflets, and transferred to plastic pots with a capacity of 14 kg, containing the same substrate used in transplanting. The pots with the young plants were taken to the greenhouse to acclimatize for a period of 45 days.

Study location

The experiment was conducted in a greenhouse belonging to Instituto de Ciências Agrárias (ICA) of the Universidade Federal Rural da Amazônia (UFRA) in Belém city, State of Pará, Brazil (01°28' 03"S, 48°29'18"W) during July until November of 2012. The biochemical analyzes were performed at the Plant Physiology Laboratory at the Universidade Federal Rural da Amazônia (UFRA) in Capitão Poço city, State of Pará, Brazil.

Experimental design

The experimental design was completely randomized with two water conditions (control and waterlogging) combined with five evaluation times (0, 4, 8, 12, and 16-days waterlogging conditions), with five replicates, and 50 experimental units in total. Each experimental unit consisted of one plant per pot.

Waterlogging application and plant treatments

After the acclimation period, all the four-month-old *P. gigantocarpa* were subjected to two water conditions and the control plants were irrigated daily with 2.5 L of water to replace the water lost by evaporation, made individually for each pot, and considering the daily weighting set (pot+plant+soil). In the treatment under waterlogging conditions, the plants were placed in pots without holes to avoid water drainage, with the water level maintained at 5 cm above the soil surface. Control and waterlogging plants remained for a period of 16 days under these conditions.

Variables analyzed

Water potential: The water potential was determined in predawn

water potential ($_p\Psi$), between 4:30 to 5:30 h, and between 10:00 to 12:00 h for the xylem water potential ($_x\Psi$), using the pressure pump Scholander (mod. PMS Instrument Co, Corvalles, USA) (Turner, 1981).

Leaf specific hydraulic conductance: The leaf specific hydraulic conductance was calculated in agreement with equation: $K_L = (g_{smd} * \Delta wmd)/(\mathrm{p}\Psi - \mathrm{md}\Psi)$, where K_L = leaf specific hydraulic conductance, g_{smd} = stomatal conductance in midday, Δwmd = variation in water saturation during midday, $_p\Psi$ = leaf water potential in predawn and $_{md}\Psi$ = leaf water potential in midday. The measurements were carried out during 0, 4, 8, 12, and 16-days of waterlogging conditions.

Nitrate concentration: Nitrate was determined with 100 mg of leaf dry matter powder incubated with 5 mL of sterile distilled water at 100°C for 30 min; after the homogenized mixture was centrifuged at 10,000 g for 15 min at 25°C and the supernatant was removed. The quantification of the nitrate was carried out at 410 nm in agreement with Cataldo et al. (1975), KNO_3 (Sigma Chemical) was used as standard.

Nitrate-reductase activity: The extraction of the nitrate-reductase enzyme was carried out with leaf disks until the weight of 200 mg was reached, the samples were incubated in 5 mL of extraction mix [0.1M KH_2PO_4, 50mM KNO_3, isopropanol at 1% (v/v) and pH 7.5] by 30 min at 30°C, and all the procedures were carried out in dark. The quantification of the enzyme activity was made by the method of Hageman and Hucklesby (1971) with absorbance at 540 nm using spectrophotometer (Quimis, model Q798DP), nitrite (Sigma Chemicals) was used as standard.

Glutamine-synthetase activity: The extraction of glutamine-synthetase enzyme was carried out with 200 mg leaf tissue ground in liquid nitrogen, the samples were incubated in 5 ml of extraction mix [Tris-HCl buffer pH 7.6 containing 10 mM $MgCl_2$. 10 mM β-mercaptoethanol, 5% (w/v) PVP and 5 mM EDTA]; after the homogenized mixture was centrifuged at 30,000 g for 10 min and the supernatant was removed. All the procedures were carried out in the interval of 0 to 4°C. The quantification of the enzyme activity was made by the method of Kamachi et al. (1991) with absorbance at 540 nm; γ-glutamylhydroxamate (Sigma Chemicals) was used as standard.

Total soluble amino acids: Determination of amino acids was performed using 50 mg of leaf dry matter powder, and was incubated with 5 ml of sterile distilled water at 100°C by 30 min, the homogenized was centrifuged to 2,000 g by 5 min at 20°C, and supernatant was removed. Quantification of the total soluble amino acids was carried out at 570 nm according to Peoples et al. (1989), L-asparagine and L-glutamine (Sigma Chemicals) were used as standard.

Proline: Proline level was determined with 50 mg of leaf dry matter poder, which was incubated with 5 ml of sterile distilled water at 100°C by 30 min after the homogenized was centrifuged to 2,000 g by 5 min at 20ºC. Quantification of proline was carried out at 520 nm according to Bates et al. (1973), in which L-proline (Sigma Chemicals) was utilized as standard.

Glycine-betaine: The determination of glycine-betaine was carried out with 25 mg of powder incubated with 2 ml of distilled water. The homogenized mixture was kept in agitation for 4 h at 25°C, after this period t was centrifuged at 10,000 g for 10 min at 25°C, and subsequently the supernatant was removed. The quantification of glycine-betaine was carried out at 365 nm in agreement with Grieve and Grattan (1983), glycine-betaine (Sigma Chemicals) was used as standard.

Alcohol-dehydrogenase and lactate-dehydrogenase activities: Enzymes alcohol-dehydrogenase and lactate-dehydrogenase were extracted from 200 mg of leaf and root tissues. Samples incubated in 2 ml of extraction mix (Tris-HCl buffer at 50mM, tiamina pirofosfato at 0.5mM, dithiothreitol at 2mM, EDTA at 1mM, NaCl at 110mM, $MgCl_2$ at 2.5 mM, with pH adjusted to 6.8). After homogenization, samples were centrifuged at 10,000 g for 10 min, and the supernatant was removed. All the procedures were carried out in the interval of 0 to 4°C. Quantification for alcohol-dehydrogenase was based on the method of Bertani et al. (1980), and lactate-dehydrogenase was based on methodology described by Hoffman and Hanson (1986), with both determinations under absorbance at 340 nm, with NADH (Sigma Chemicals) as a standard.

Data analysis

The data were subjected to variance analysis and significant differences between means were determined by F test at 5% level of error probability (Steel et al., 2006). The standard deviations were calculated to each treatment in all evaluation points. The correlation analysis was performed by the Pearson parametric method, and the statistical procedures were carried out with the SAS software (SAS Institute Inc, 2008).

RESULTS

Water potential (Ψ) in plants subjected to waterlogging

In assessing the $_p\Psi$ and $_x\Psi$ it was observed significant difference between treatments (P≤0.001) and between the evaluated periods (P≤0.001) further showing interaction between the factors (P≤0.001) (Figure 1). The $_p\Psi$ (4:30-5:30 h) in the control plants subjected to waterlogging conditions was -0.28 and -0.40 MPa, respectively, giving a decrease of 42.85% in plants in waterlogged conditions compared to the control (Figure 1A). In times of 10:00 to 12:00 h the $_x\Psi$ was -0.84 and -1.69 MPa, respectively, resulting in decrease of 101.19% in plants subjected to waterlogging compared to the control (Figure 1B).

Influence of waterlogging conditions on hydraulic conductivity of *P. gigantocarpa* plants

Figure 2 showed a significant interaction between treatments (P≤0.001) and periods of exposure to stress (P≤0.05), as well as the interaction between the factors (P≤0.001). In control and flooded plants, the values obtained were 0.29 and 0.02 mol m^{-2} s^{-1} Mpa^{-1}, respectively, thus, the plants grown in the absence of oxygen conditions showed a decrease of 93.1% compared to control plants.

Interference induced by waterlogging conditions on nitrate concentration in young plants

The analysis of the nitrate concentration in young plants

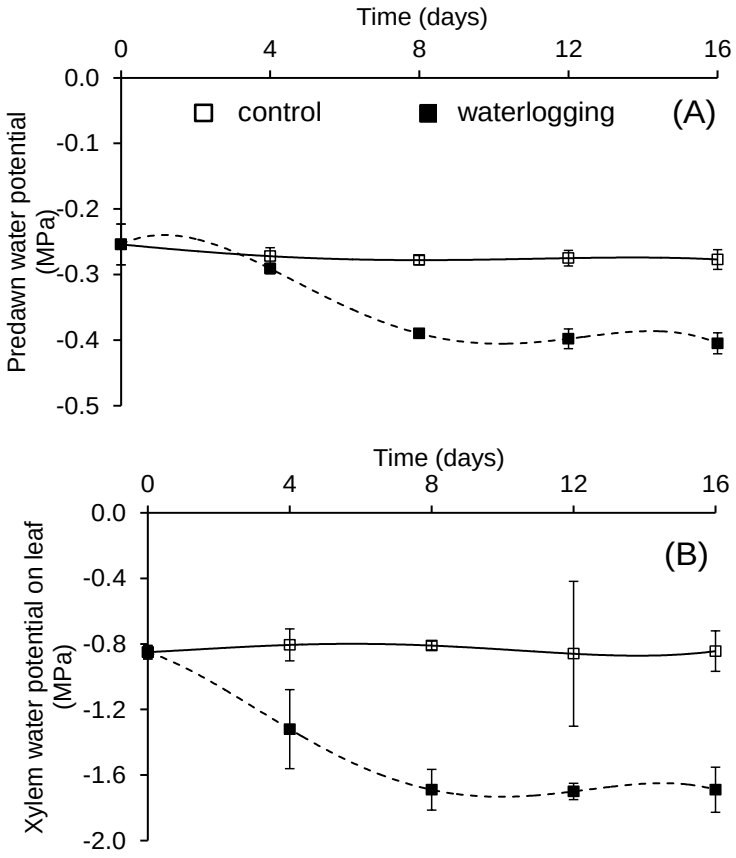

Figure 1. Predawn water potential (A) and xylem water potential of the leaf (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

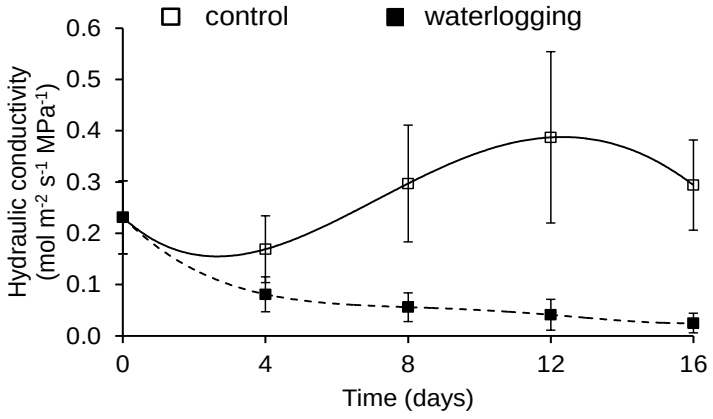

Figure 2. Hydraulic conductivity in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

of *P. gigantocarpa* showed significant differences between treatments (P≤0.001) and periods of exposure to stress (P≤0.001) as well as the interaction between the factors (P≤0.001) (Figure 3). In plant leaves from control

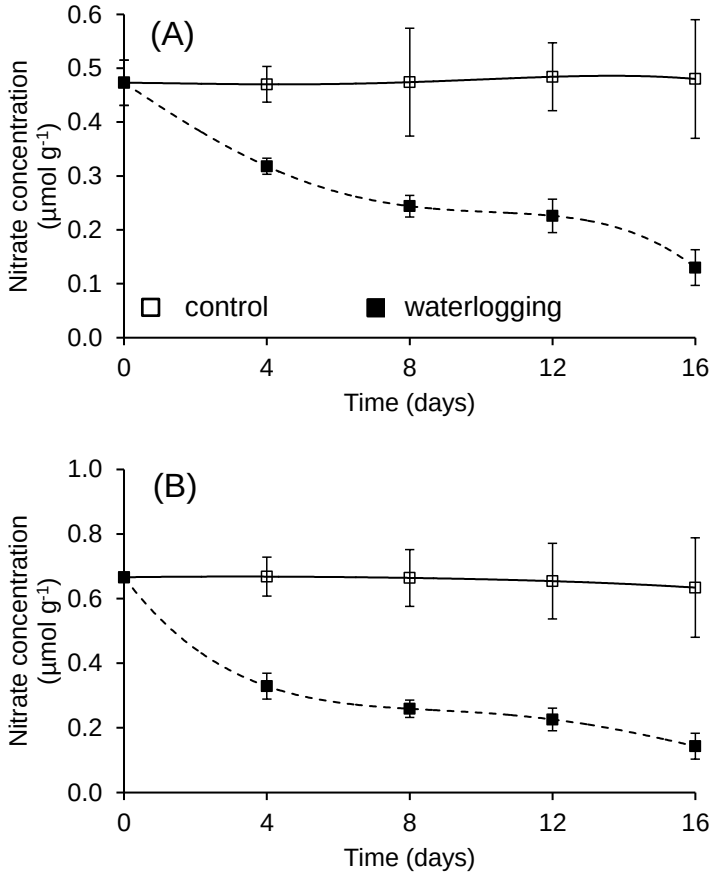

Figure 3. Nitrate concentration in the leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

and waterlogging conditions the values obtained were 0.48 and 0.13 µmoles g^{-1} of $N0_3^-$, respectively (Figure 3A). While, in the root system the nitrate concentrations were 0.63 and 0.14 µmoles g^{-1} of $N0_3^-$ the control and flooded plants, respectively (Figure 3B). Thus, though the results showed a decrease of 72.92 and 77.80% in plants subjected to waterlogging conditions compared to control plants.

Effect of waterlogging conditions on nitrate-reductase activity (NRA) in leaves and roots of *P. gigantocarpa*

The nitrate-reductase activity was significantly decreased in the flooded plants, regardless of the sampled vegetative parts, thus, the absence of oxygen promoted reduction of the nitrate-reductase activity in both leaves and roots of young plants of *P. gigantocarpa* (Figure 4). The values obtained for the NRA variable showed significant differences between treatments (P≤0.001) and periods of exposure to stress (P≤0.001), as well as the interaction between the factors (P≤0.001). In plant leaves from control and waterlogging conditions, the values obtained were 0.83 and 0.58 µmoles g^{-1} of NO_2^-, respectively, thus, showing reduction of 30.12% in plants subjected to waterlogging conditions compared to control plants (Figure 4A). In the roots, the values were 0.85 and 0.63 µmoles g^{-1} of NO_2^-, respectively, resulting in reduction of 25.88% in the waterlogging conditions compared to the control plants (Figure 4B).

Modifications produced by waterlogging conditions on glutamine-synthetase (GS) of *P. gigantocarpa* plants

The waterlogging conditions significantly reduced the activity of glutamine-synthetase in leaves and roots of flooded plants (Figure 5). The values obtained for GS variable showed significant difference between treatments (P≤0.001), and the periods of exposure to

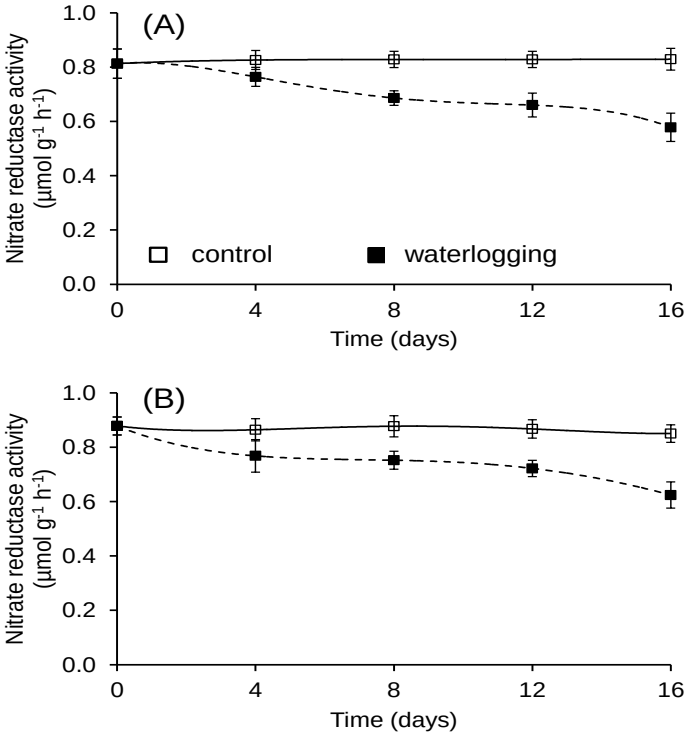

Figure 4. Nitrate-reductase activity in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

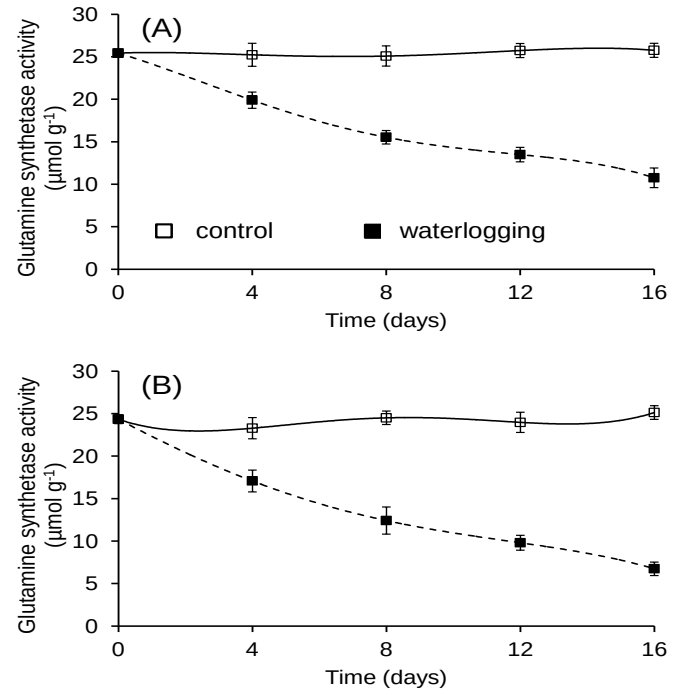

Figure 5. Glutamine-synthetase in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

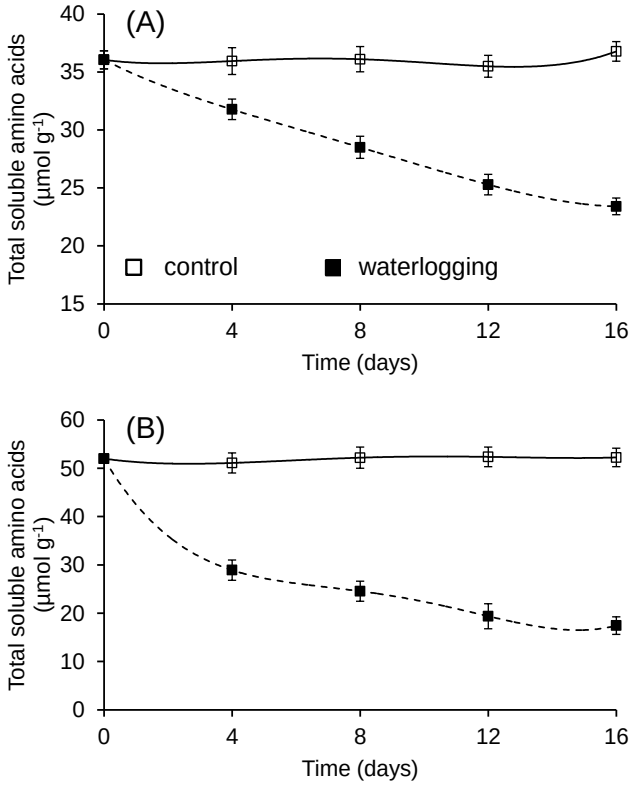

Figure 6. Total soluble amino acids in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

stress (P≤0.001), as well as the interaction between factors (P≤0.001). In leaves of control and flooded plants, the concentration of glutamine-synthetase was 27.8 and 10.8 mmoles kg^{-1} of GGH, respectively, corresponding to a 61.15% reduction in waterlogging conditions compared to control plants (Figure 5A). In the roots, the concentration of glutamine-synthetase was 25.1 and 6.7 mmoles kg^{-1} de GGH in control and flooded plants, respectively, with a decrease of 73.3% in the waterlogging conditions (Figure 5B).

Impact of waterlogging conditions on total soluble amino acids in plants

The total soluble amino acid concentrations in leaves and roots of young plants of *P. gigantocarpa* varied significantly between the treatments (Figure 6). The evaluations showed significant differences between the treatments (P≤0.001), and periods of exposure to stress (P≤0.001), as well as the interaction between the factors (P≤0.001). The analysis of the total soluble amino acid in the leaves of the control plants showed a concentration of 36.8 µmol g^{-1} of amino acid, while the plants subjected to waterlogging conditions provided value of 23.4 µmol g^{-1} of amino acid (Figure 6A). By the information presented in Figure 6A, a decrease of 93.47% in plants subjected to waterlogging conditions compared to the control plants was observed. In the roots of young plants of *P. gigantocarpa* were observed values 52.23 and 17.45 µmol g^{-1} of amino acid in control and flooded plants, respectively, representing a decrease of 66.59% in plants subjected to waterlogging conditions compared with the control plants (Figure 6B).

Effects on proline in plants subjected to waterlogging conditions

By the information presented in Figure 7, increase in proline concentrations in leaves and roots of plants subjected to waterlogging conditions was observed. The results showed significant differences between the treatments (P≤0.001), and periods of exposure to stress (P≤0.001), as well as the interaction between the factors (P≤0.001). In leaves of control and flooded plants the concentrations were 1.37 and 5.16 µmol g^{-1} of proline, respectively, an increase of 276.64% of proline in plants

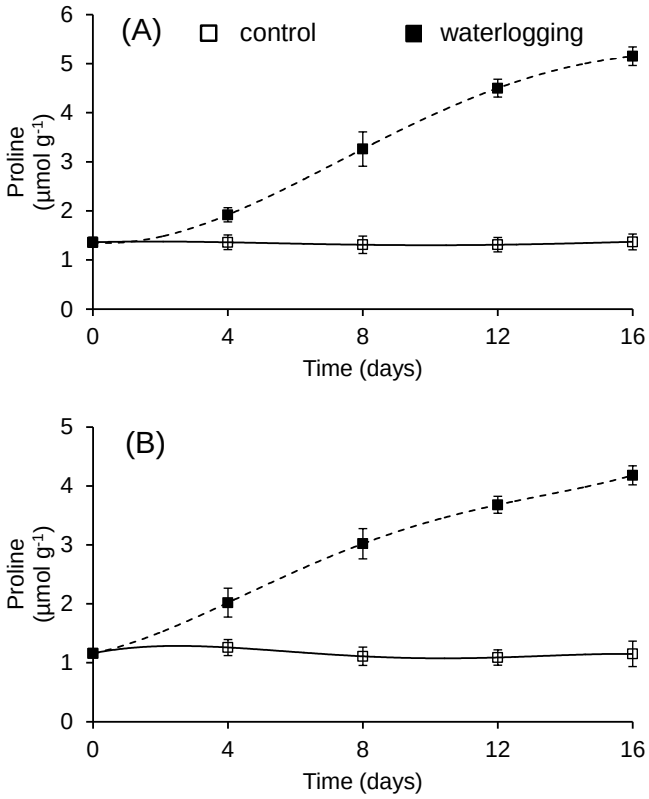

Figure 7. Proline in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

subjected to waterlogging conditions compared the control plants (Figure 7A). While, in the roots of young plants of *P. gigantocarpa* were obtained values of 1.52 µmol g^{-1} of proline in control plants, and 4.19 µmol g^{-1} of proline in plants subjected to waterlogging conditions, an increase of 175.6% in plants subjected to waterlogging conditions compared to control plants (Figure 7B).

Effects promoted by waterlogging conditions on glycine-betaine of *P. gigantocarpa* plants

For the variable glycine-betaine results showed significant differences between the treatments (P≤0.001), and periods of exposure to stress (P≤0.001), as well as the interaction between the factors (P≤0.001) (Figure 8). The evaluation of the concentration of glycine-betaine in the leaves of control and flooded plants showed values of 4.78 e 15.19 µg g^{-1} of glycine-betaine, respectively, thus, an increase of 217.78% in plants subjected to waterlogging conditions were compared to control plants (Figure 8A). For the roots, the values obtained were 6.57 and 15.29 µg g^{-1} of glycine-betaine in the control and flooded plants, respectively, thus, an increase of 132.72% in plants subjected to waterlogging conditions compared to control plants (Figure 8B).

Interference induced by waterlogging conditions on alcohol-dehydrogenase (ADH) and lactate-dehydrogenase activity (LDH) in young plants of *P. gigantocarpa*

The absence of oxygen significantly affected the activity of ADH (Figure 9) and LDH (Figure 10) of young plants of *P. gigantocarpa*, both the aerial part and the root system. The analysis of the activity of enzymes dehydrogenase alcohol-dehydrogenase and lactate-dehydrogenase activity showed significant differences between treatments (P≤0.001), and periods of exposure to stress (P≤0.001), as well as the interaction between the factors (P≤0.001). In the leaves of ADH, activity was 0.06 and 2.88 moles of NADH H^+ kg^{-1} of protein min^{-1} in control and flooded plants, respectively, with an increase of 97.92% compared to the control plant (Figure 9A). In the roots of the enzyme activity in the control and flooded plants were 2.6 and 12.0 moles of NADH H^+ kg^{-1} of protein min^{-1}, respectively, corresponding an increase of 361.54% in

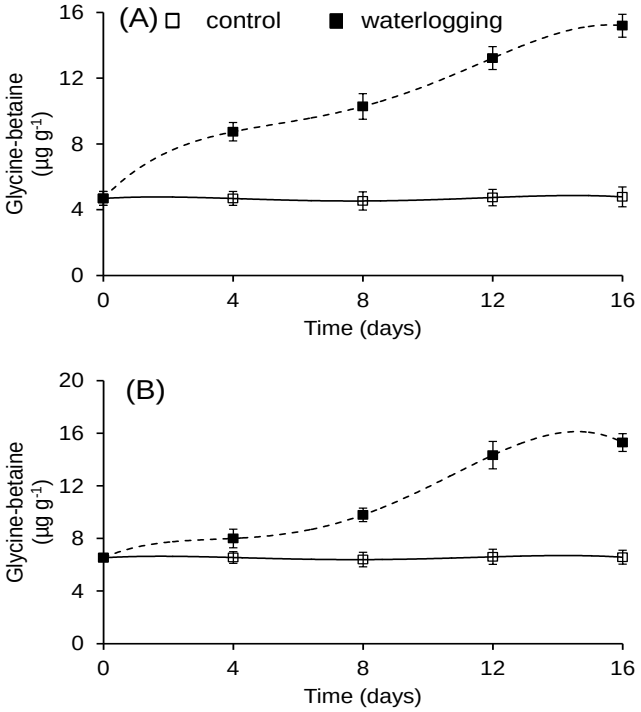

Figure 8. Glycine-betaine in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

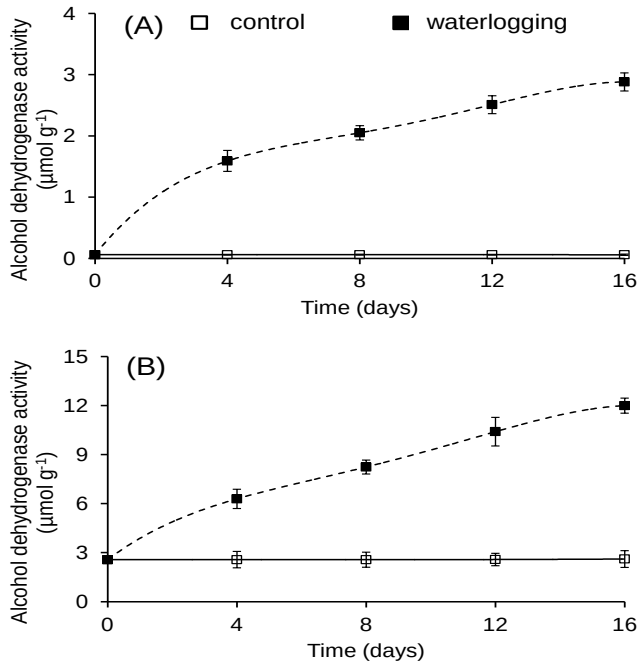

Figure 9. Alcohol-dehydrogenase activity in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

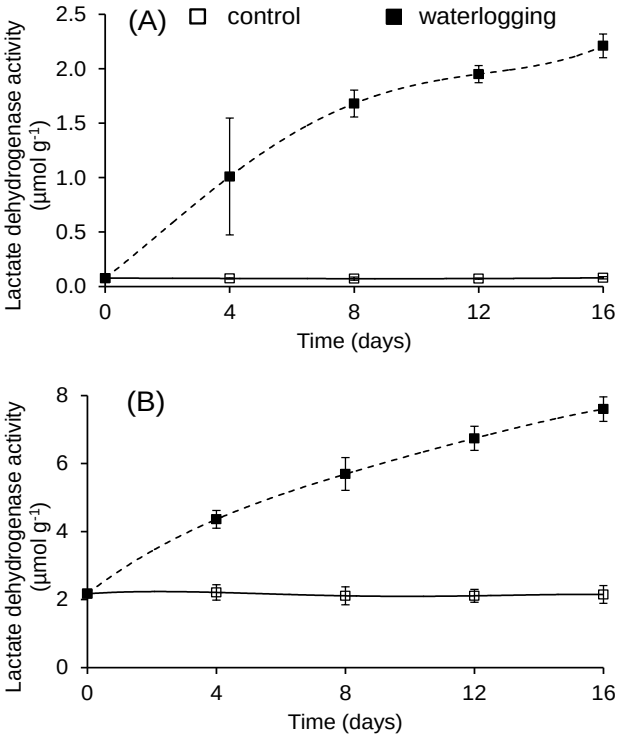

Figure 10. Lactate-dehydrogenase activity in leaf (A) and the root (B) in young plants of *P. gigantocarpa* subjected to waterlogging conditions. Squares represent the mean values of five replicates, and error bars represent the mean standard errors.

plants subjected to waterlogging conditions compared to control plants (Figure 9B). The LDH activity in leaves were 0.08 and 2.21 moles of NADH H^+ kg^{-1} of protein min^{-1} in control and flooded plants, respectively, with a 96.38% increase compared to the control plant (Figure 10A). The roots values obtained were 2.16 and 7.6 moles of NADH H^+ kg^{-1} of protein min^{-1} in control and flooded plants, respectively, thus, an increase of 71.58 % was observed in plants subjected to waterlogging conditions compared to control plants (Figure 10B).

DISCUSSION

The reduction of the root system of young plants of *P. gigantocarpa*, contributed in reducing $_x\Psi$, which continued until the 8th day of waterlogging conditions (Figure 1B). This period observed relative stabilization of $_x\Psi$. This can be explained by the appearance of hypertrophic lenticels, which improves aeration of the roots and minimizes decrease in $_x\Psi$. Folzer et al. (2006) studying in young plants of *Quercus petraea* in waterlogged conditions for 14 days, found out that the increased in $_x\Psi$ in flooded plants, coincided with the appearance of hypertrophic lenticels after 10 days of water saturation. Islam et al. (2010) studying the effect of waterlogged conditions in young plants of two genotypes of *Vigna radiata* (GK48 e BARImung5), observed a significant reduction of $_x\Psi$ in flooded plants compared to control plants. These authors attributed the smaller reduction of $_x\Psi$ in the GK48 genotype production of a bigger amount of adventitious roots. In this study, the relative stabilization of $_x\Psi$ was not influenced by the formation of adventitious roots, since the reduction in the decrease of $_x\Psi$ began on the 8th day of waterlogging conditions (Figure 1B), in which the adventitious roots were identified in the last evaluation (16th day of waterlogged conditions).

Through the information presented in Table 1, negative correlation was observed ($P \leq 0.001$) between $_x\Psi$ with proline and glycine-betaine. Thus, the increase of compatible osmolytes, possibly, contributed to reduce the water potential of the plant tissue, indicating a possible occurrence of osmotic adjustment in young plants of *P. gigantocarpa*. Gimeno et al. (2012) studying young plants of *Jatropha curcas* L. submitted the waterlogged conditions for 10 days, observed a significant reduction in $_x\Psi$ of flooded compared to the control plant, as a result of plant subject to waterlogged conditions did not increase the concentration of proline. Similar results of $_x\Psi$ were

Table 1. Pearson correlation coefficient between transpiration (E), stomatal conductance (gs), foliar xylem water potential (xΨ) and hydraulic conductivity (K_L) in young plants of *P. gigantocarpa* subjected to waterlogging conditions.

Conditions		K_L	Ψ_x	gs
Control	E	0.1597^{ns}	0.0904^{ns}	0.4117*
	gs	0.4900*	-0.0192^{ns}	-
	$_x\Psi$	0.3509^{ns}	-	-
Waterlogging	E	0.7243**	0.7020**	0.9538**
	gs	0.7618**	0.6922**	-
	$_x\Psi$	0.8368**	-	-

^{ns} Not significant by F test, * Significant by F test (P≤0.05), ** Significant by F test (P≤0.01).

Table 2. Pearson correlation coefficient between transpiration (E), the foliar xylem water potential ($_x\Psi$), hydraulic conductivity (K_L), stomatal conductance (gs), relative humidity (RH), photosynthetically active radiation (PAR), leaf temperature (T_{leaf}), air temperature (T_{air}), vapor pressure deficit between leaf and air (VPD_{la}), proline on the leaf (P_{leaf}) and root (P_{root}), glycine-betaine on the leaf (GB_{leaf}) and the root (GB_{root}), and number of leaves (NL) in young plants of *P. gigantocarpa* subjected to waterlogging conditions.

Conditions		E	$_x\Psi$	K_L	gs
Control	RH	0.1158^{ns}	0.2694^{ns}	- 0.5519**	- 0.0484^{ns}
	PAR	0.1617^{ns}	- 0.1855^{ns}	0.1288^{ns}	0.4695*
	T_{leaf}	- 0.0047^{ns}	- 0.2190^{ns}	- 0.0576^{ns}	- 0.4409*
	T_{air}	- 0.0006^{ns}	- 0.2181^{ns}	- 0.0554^{ns}	- 0.4353*
	VPD_{la}	- 0.0824^{ns}	- 0.2870^{ns}	0.5221**	- 0.0107^{ns}
	P_{leaf}	0.0460^{ns}	0.1586^{ns}	0.2223^{ns}	- 0.1230^{ns}
	P_{root}	0.0927^{ns}	0.1135^{ns}	- 0.0586^{ns}	0.1172^{ns}
	GB_{leaf}	- 0.4632**	- 0.1214^{ns}	- 0.0909^{ns}	- 0.3221^{ns}
	GB_{root}	0.1758^{ns}	- 0.1670^{ns}	0.3073^{ns}	- 0.0416^{ns}
	NL	0.0281^{ns}	-	-	0.2238^{ns}
Waterlogging	RH	0.3728^{ns}	0.1589^{ns}	- 0.1579^{ns}	0.3574^{ns}
	PAR	0.1623^{ns}	0.1247^{ns}	0.2004^{ns}	0.0504^{ns}
	T_{leaf}	- 0.0029^{ns}	0.2711^{ns}	0.4655*	0.0048^{ns}
	T_{air}	- 0.0053^{ns}	0.2688^{ns}	0.4627*	0.0004^{ns}
	VPD_{la}	- 0.3248^{ns}	- 0.1022^{ns}	0.2125^{ns}	-0.3130^{ns}
	P_{leaf}	- 0.8569**	- 0.8049**	- 0.7095**	-0.8180**
	P_{root}	- 0.8270**	- 0.8525**	- 0.7706**	-0.8002**
	GB_{leaf}	- 0.8115**	- 0.8356**	- 0.7928**	-0.7716**
	GB_{root}	- 0.8192**	- 0.7339**	- 0.6690**	-0.7881**
	NL	0.4860*	-	-	0.4584*

^{ns} Not significant by F test, * Significant by F test (P≤0.05), ** Significant by F test (P≤0.01).

described by Alves et al. (2012) in *Tabebuia serratifolia* (Vahl) Nicholson submitted to waterlogged conditions for 9 days, in which they observed values of -2.3 MPa in plants subjected to waterlogged conditions.

The hydraulic conductivity of the root was significantly correlated with the $_x\Psi$, stomatal conductance and transpiration plant (Table 2). Thus, the decrease in hydraulic conductivity possibly influenced the reducing of $_x\Psi$, and stomatal closure of *P. gigantocarpa* in order to avoid the internal water deficit. Then, the hydraulic conductivity worked as a corregulador while the $_x\Psi$, stomatal conductance and plant transpiration in plants

were subjected to waterlogged conditions.

The young plants of *P. gigantocarpa* showed significant reduction of nitrate-reductase activity, in the roots and the leaves, with increasing time of exposure to waterlogged conditions. Possibly, the water saturation in the soil caused an increase of inorganic phosphate, as a function of the reduction of ATP, promoting the phosphorylation of nitrate reductase, providing its connection with the 14-3-3 protein, resulting in decreased enzyme activity in the plant. The results obtained for the nitrate-reductase activity in young plants of *P. gigantocarpa* corroborate with the studies described by Alves et al. (2012), in which they observed a reduction of this enzyme in roots and leaves of *Tabebuia serratifolia* (Vahl) Nicholson subjected to waterlogged conditions for 9 days.

For the induction of nitrate-reductase activity, it required the presence of NO_3^-, thus, the nitrate-reductase activity is regulated by NO_3^- metabolism located in the cytoplasm (Allègre et al., 2004). The decrease in the concentration of NO_3^- with the period of exposure to waterlogged conditions of young plants of *P. gigantocarpa* limited the nitrate-reductase activity (Figures 3 and 4), affecting the assimilation of NO_3^- and influenced on nitrogen metabolism, following the reduction in glutamine-synthetase (Figure 5) amino acids at the root and the leaves of the plants.

The reduction of nitrate-reductase activity in leaves of young plants of *P. gigantocarpa* subjected to waterlogged conditions, is related to low translocation of NO_3^- from the root (Alaoui-Sosse et al., 2005), demonstrating the dependence of nitrate-reductase by NO_3^- carried in the transpiration stream, which in this study was affected, probably, by reducing the water potential of the leaf xylem (Figure 1B) of stomatal conductance and hydraulic conductivity (Figure 2) in young plants of *P. gigantocarpa* subjected to waterlogged conditions. However, the reduction of the nitrate-reductase activity in flooded plants is related to the increase in alcoholic fermentation, evidenced by the increase in the ADH activity (Figure 9), the resulting pH increase, thus, inhibiting the activity of nitrate-reductase.

The reduced activity of glutamine-synthetase in the flooded plants, is related to the reduced availability of ATP, since this enzyme is strongly dependent on energy derived from ATP phosphorylated produced in the glycolytic pathway, in mitochondrial oxidative phosphorylation and during the photosynthetic activity in leaves. In *T. serratifolia,* plants were subjected to waterlogged conditions, the activity of this enzyme in the roots and leaves, significantly reduced compared to the activity in plants in aerobic conditions (Alves et al., 2012). The authors attributed the decrease in activity of this enzyme, and reduced synthesis of ATP in the cell tissues.

Another justification for the reduction of the glutamine-synthetase activity in plants subjected to waterlogged conditions is the decrease in glutamate synthase and, of glutamate, in the chloroplast or the cell plastids (Horchani and Aschi-Smiti, 2010), or the reduction of the nitrate-reductase activity, caused by the ion NH_4^+ limitation (Alves et al., 2012). The main pathway of uptake of NH_4^+ in plants occurs by means of glutamine-synthetase activity, which is dependent of ATP, acting as a catalyst of binding of NH_4^+ with glutamic acid to form glutamine (Masclaux-Daubresse et al., 2010), this amino acid is the main source of organic nitrogen transported from roots to leaves through the xylem to the biosynthesis of all nitrogenous compounds (Okumoto and Guillaume, 2011), justifying the results obtained in this study, in which there was an observed reduction in glutamine-synthetase activity in plants *P. gigantocarpa* subjected to waterlogged conditions (Figure 5), simultaneously with NH_4^+ accumulation and reducing nitrogen compounds, such as amino acids. In plants of *S. lycopersicum* subjected to waterlogged conditions, the activity of glutamine-synthetase was significantly inhibited in the leaves, together with the increase of concentration of NH_4^+ in the plant tissue compared with plants grown under aerobic conditions (Horchani and Aschi-Smiti, 2010). According to these authors, the reduction in glutamine-synthetase activity in the leaves of *S. lycopersicum* plants is related to a reduction of glutamate, which was used in the synthesis of proline and glycine-betaine, as an alternative for maintaining the metabolism and osmotic adjustment.

The glutamine-synthetase is an enzyme precursor in the formation of all amino acids at the root and leaves of higher plants (Taiz and Zeiger, 2013), therefore, in this study the young plant of *P. gigantocarpa* decreased the glutamine-synthetase activity (Figure 5), justifying the reduction of total soluble amino acids in the leaves and roots of *P. gigantocarpa* plant (Figure 6). Alves et al. (2012) observed that decreased glutamine-synthetase activity promoted reduction of 87.6% of amino acids in roots and 76.2% in leaves of young plants *T. serratifolia* subjected to waterlogged conditions for 9 days.

Another possible explanation for the reduction of amino acids is due to waterlogged conditions which has promoted the reduction in ATP synthesis, resulting in a decrease in the absorption of nitrate, or, according to Kreuzwieser et al. (2009) the reduction of amino acids in plants subjected to waterlogged conditions is due to dynamic changes of the transcriptional levels that encode enzymes involved in its metabolism. In Table 1, a significant positive correlation coefficient ($P<0.001$) between the proline and the concentration of ammonium was observed. In plants subjected to waterlogged conditions, the increased ammonium concentration, or protein breakdown by proteolytic enzymes, possibly, was responsible for the high concentration of free proline, in which it is important in the protection of cellular structures against oxidative damage caused by free radicals (Kavi-Kishor et al., 2005). Furthermore, the increase in proline may have acted as carbon and nitrogen source for plant

growth (Silva-Ortega et al., 2008) or, in which it is an osmoprotectors amino acid, assisted in reducing the water potential of tissues, reducing dehydration. The results obtained in *P. gigantocarpa* corroborate with the studies by Horchani et al. (2010) and Parvin and Karmoker (2013) observed an increase of proline in roots and leaves of plants of *S. lycopersicume* and *C. capsularis* L. subjected to waterlogged conditions.

In the present study, a significant negative correlation (P≤0.001) between the glycine-betaine and the $_{x}\Psi$ in plants subjected to waterlogged conditions (Table 1) was observed. The increase of glycine-betaine in leaves and roots is probably related to osmotic adjustment hyaloplasm plants subjected to stress (Jaleel et al., 2007), thus, the accumulation of glycine-betaine enable the reduction of cellular water potential during periods of osmotic stress (Taiz and Zeiger, 2013), favoring the absorption and soil water transport to aerial part of the plant, thereby, protecting the plant tissues and physiological processes, improving plant tolerance to abiotic stress. Another factor that contributes to the increase in glycine-betaine concentrations was the synthesis of amino acids due to the breakdown of proteins and increased ammonia concentrations promoted by photorespiration (Colmer et al., 2009).

The high activity of ADH with the exposure period to waterlogged conditions (Figure 9), demonstrates that *P. gigantocarpa*, requires, among other mechanisms, rapid and permanent use of fermentative pathway metabolism as a way of maintaining, regenerating the reducing power and ATP production, having an efficient anaerobic respiration when subjected to lack of oxygen in roots. The high activity of LDH (Figure 10), and consequently, the signaling mechanism is required to initiate or promote the activity of ADH in the stimulation of ethanolic fermentation, by consuming more protons than lactic, increasing the cytosolic pH survivability of plants subjected to waterlogged conditions (Dolferus et al., 2008), which explains the results obtained in this study.

The results obtained in this study, the increase of ADH and LDH activity in leaves and roots is probably a strategy for continued growth and survival of young plants of *P. gigantocarpa* under waterlogging conditions. Furthermore, the synthesis of enzymes ADH and LDH act as an alternative to compensate the depletion of proteins (Zabalza et al., 2009) in which it occurs during oxygen deficiency.

Conclusions

The waterlogged conditions adversely affect the water potential, hydraulic conductivity, concentration of nitrate, nitrate-reductase activity, glutamine-synthetase and concentration of total soluble amino acids in young plants of *P. gigantocarpa*. In contrast, the concentrations of proline, glycine-betaine and the activities of alcohol-dehydrogenase and lactate-dehydrogenase showed an increase in plants grown under conditions of absence of oxygen. Thus, the young plants of *P. gigantocarpa* cannot be recommended for cultivation in waterlogged conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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