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African Journal of Agricultural Research

19 July, 2018
ISSN 1991-637X
DOI: 10.5897/AJAR
www.academicjournals.org

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African Journal of Agricultural Research

Table of Contents: Volume 13 Number 29, 19 July, 2018

ARTICLES

Vol. 13(29), pp. 1460-1467, 19 July, 2018
DOI: 10.5897/AJAR2018.13226
Article Number: 423670757844
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Thorn apple (*Datura stramonium* L.) allelopathy on cowpeas (*Vigna unguiculata* L.) and wheat (*Triticum aestivum* L.) in Zimbabwe

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Received 5 May, 2018; Accepted 5 June, 2018

***Datura stramonium* extracts have allelopathic properties. The study was conducted to investigate the allelopathic effects of *D. stramonium* weed on seed germination, early seedling growth and dry biomass of crop plants (*Triticum aestivum* and *Vigna unguiculata)*. Laboratory and greenhouse trials were arranged as completely randomised design and the field pot experiment was arranged as a randomised complete block design. Aqueous leaf extracts of *D. stramonium* at 2, 4, 6 and 8% concentrations were applied to determine their effects on seed germination, early seedling growth of crops under laboratory, field and greenhouse conditions. Distilled water (0%) acted as a control. Results from the study indicated that germination, shoot length and dry weight significantly decreased proportionally ($p<0.001$) as the concentration increased from 2 to 8%. The results showed that *D. stramonium* has allelopathic effects on wheat and cowpeas, hence cannot be used as a bio herbicide to control *Tagetes minuta* and *Amaranthus hybridus* on the selected crops since it is non selective to the crops studied. There is therefore need for further research on screening of arable crops against the allelopathic effects of *D. stramonium.* This will help to identify arable crops which are not negatively affected by allelochemicals from *D. stramonium* weed so that it can be used as a selective bio herbicide against other weeds.**

Key words: Allelopathy, aqueous leaf extract*, Datura stramonium, Triticum aestivum, Vigna unguiculata*.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is an important food legume and an essential component of cropping systems in the drier and marginal areas of the tropics and subtropics (Fatokan et al., 2000). Cowpea is a summer annual legume that grows under extreme drought conditions. It is grown for vegetable or grain consumption (Saidi et al., 2007) and has numerous advantages ranging from high nutrition (25% protein, 1.4% fat, 60.8% carbohydrates and 3.4% ash), soil fertility improvement and weed control (Aliyu and Emechebe, 2003).

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Wheat (*Triticum aestivum* L.) is grown throughout the world and is adaptable to the wide range of environmental conditions. Wheat is used for human and livestock consumption. In Zimbabwe, it is mainly used as human food in the form of bread. Wheat is used in the making of pasta products, breakfast cereals, cake and tortilla.

Allelopathy involves harmful or beneficial effects of one plant upon another through the production of secondary chemical compounds that technically escape into the environment in sufficient quantity and with enough persistence to cause the enrolled effects (Khalid et al., 2002; Thi et al., 2015). Allelochemicals emancipated as residues, exudates and leaches by many plants from leaves, stem, roots, fruits and seeds were reported to interfere with the growth of other plants (Asgharipour and Armini, 2010). Allelopathy in this era is recognized as appropriate potential technology to control weeds using chemicals released from decomposed plant parts of other species (Naseem et al., 2009).

If allelopathy is to be exploited in weed management then information has to be generated on the effects of the allelochemicals on food crops. Thakur and Bhardwai (1992) reported that leachates from *Eucalyptus globulus* leaves significantly reduced maize germination but were ineffective on wheat germination. Studies have also demonstrated the harmful influence of the application of *Eucalyptus camaldulensis* plant extracts to sorghum seed germination, seedlings emergence and biomass gain (Mohamadi and Rajaie, 2009). Aqueous extracts of leaves notably inhibited seed germination of sorghum with application of *Parthenium hysterophorus* (Murthy et al., 1995), *Ipomoea carnea*, *Commelina benghalensis* and *Cyprus rotundus* (Channappagoudar et al., 2003) and *Eucalyptus camaldulensis* (Mohamadi and Rajaie, 2009). However, these allelochemicals sometimes have positive effects on sorghum growth; *Moringa oleifera* leaf extracts enhanced germination of sorghum by 29% (Phiri, 2010). The same kind of germination promotion behaviour was also observed in extract of *Cassia angustifolia* (Hussain et al., 2007).

Thorn apple (*D. stramonium*) is an annual poisonous plant that grows to approximately 1.5 m high. It is characterised by solitary white, trumpet-shaped flower (Fatoba et al., 2011). It has been said that several chemicals have been identified and phytochemical investigators believe that there are still many other chemicals which have not been identified to be exploited as bioherbicides (Elisante and Ndakidemi, 2014). Allelochemicals found in *D. stramonium* have allelopathic effects on survival of native plants. *D. stramonium* contains a series of allelochemical in form of alkaloids, atropine, hiosciamine and scopolamine (Butnariu, 2012), which inhibits the growth and development of root and shoots of *Trigonella* and *Lepidium* in a concentration dependent manner (EL-shora and Abd EL-Gawad, 2014, EL-shora et al., 2015a; An et al., 1996). These allelochemicals are said to reduce cell division or auxin that induces the growth of shoot and root (Gholami et al., 2011). Furthermore allelochemicals affect the root system of the plant through reduction in shoot extension, number of roots, curling of the root axis, swelling or necrosis of root tips and lowered reproductive capacity of the plant (An et al., 1998). Inhibition of growth caused by these allelochemicals may probably be due to its interference with the plant growth processes (Gholami et al., 2011).

There is limited research on *D. stramonium* allelopathy on arable crops. Therefore, the objective of this study was to determine *D. stramonium* allelopathy on *V. unguiculata* and *T. aestivum*.

MATERIALS AND METHODS

Experiment 1: Effects of *D. stramonium* concentration on the germination and early establishment of *T. aestivum* and *V. unguiculata*

Study site

The laboratory experiment was carried out at Midlands State University, located in Midlands province of Zimbabwe. The geographical location is 19°45' S (line of latitude) and 29°85' E (line of longitude). The site is in agro-ecological region III, at an altitude of 1428 m. The mean annual temperature was 18°C.

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times. Treatments were 20 ml of distilled water (control) and aqueous *D. stramonium* applied at 2, 4, 6 and 8% concentration as a ratio of plant extract powder to 100 ml distilled water. Two grams of extract powder was added to 100 ml of distilled water to give 2% concentration of aqueous and similarly, 4, 6 and 8% concentrations were prepared.

Preparation of concentrations

Leaves of fully grown plants were washed to remove soil particles. The material was then cut into pieces and shade dried for one month. After drying, the material was crushed into powder form manually using a traditional mortar and pistil. Further grinding was done by using an electric mortar.

The material (powder and distilled water) was mixed and poured into a conical flask with its mouth closed and kept for 24 h in the dark at room temperature according to the method used by Dhavan and Narwal (1994). The four flask were marked with stickers according to the *D. stramonium* concentrations (2, 4, 6 and 8%). This was followed by filtration process in two steps, in the first step muslin cloth was used and later the filtrate was allowed to pass through Whatman filter paper No. 1. The prepared aqueous concentrations were kept in a refrigerator to prevent conversions of some of the compounds upon exposure to light and high temperature.

Experimental procedure

Two hundred and twenty five seeds of the wheat and cowpeas were surface sterilized with 0.1% mercuric chloride solution for

2 min and washed twice with distilled water. The petri dishes were labeled with a permanent marker in relation to concentration level. Fifteen seeds of each weed were placed in petri dishes on Whatman filter paper No. 1. 20 ml of each *D. stramonium* aqueous concentration (2, 4, 6 and 8%) was added to each Petri dish. 20 ml of distilled water was used as a control. Watering was done after every three days and the petri dishes were kept in an incubator at 24°C room temperature for 10 days.

Data collection

Seed germination was recorded on the 7th day; seeds with emerged radical were counted and recorded. Seedling plumule length and radicle length was also measured using a 30 cm ruler on the 10th day after sowing in the petri dishes.

Experiment 2: Pot experiment: Effects of different *D. stramonium* aqueous concentrations on germination and early seedling growth of weeds in the field

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times and two crops were tested.

Experimental procedure for the field and greenhouse experiments

Two hundred and twenty five seeds of the selected crops were surface sterilized with 0.1% mercuric chloride solution for 2 min and washed twice with distilled water. Five litres pots were used and they were filled with mixtures of soil (loamy sand). Fifteen seeds of each of the tested weeds were sown in each pot at 0.5 cm and then irrigated with various solutions to field capacity every three days

Data collection for the field and greenhouse experiments

Data on seed emergence, shoot, and root length; seedling fresh and dry weight was recorded. Seed emergence was determined by physically counting the number of seedlings on the 8th day after planting. During the experiment period (after 30 days after planting), shoot and root length was also measured using a 30 cm ruler. The dry weight was determined by placing the tested samples in the oven to a temperature of 110°C for 48 h until a constant weight was realised.

Experiment 3: Effects of different *D. stramonium* aqueous concentrations on germination and early seedling growth of crops and weeds in the greenhouse

Study site

The greenhouse experiment was carried out at Morningside in Masvingo Province of Zimbabwe at a geographical location of latitude 20° 7' 17S and longitude 30° 49' 58 E. The site is in agro-ecological zone 4, at an altitude of 1034 m above the sea level. It receives an average of 600 mm of rain annually with a mean annual temperature of 28°C.

Experimental design

The experiment was arranged as a complete randomized design with five treatments replicated 3 times. Sixty pots were used and each replication had twenty pots. Two hundred and twenty five seeds of the selected crops were surface sterilized with 0.1% mercuric chloride solution for 2 min and washed twice with distilled water. Five litres pots were used, filled with mixtures of soil (loamy sand). Fifteen seeds of each of the tested weeds and crops were sown in each pot at 0.5 cm depth, and then irrigated to field capacity every 3 days with plant extracts at 2, 4, 6, and 8%.

Data analysis

Collected data was subjected to Analysis of Variance at 5% significance level using Genstat 4.0 version 2013. Fishers protected least significance test at 5% was used to separate the means where significant differences were noted.

RESULTS

The results show that there was a significant $P<0.001$ of the leaf extract concentration with water having the highest germination percentage, radicle length, root length, plumule length, shoot length, fresh and dry biomass of wheat and cow peas as compared to the rest of the treatments in the laboratory, field and greenhouse.

Seed germination and emergence

The results showed that an increase in thorn apple concentration led to the decrease in germination and emergence as shown in Figures 1 to 3. The highest germination (100) was recorded where distilled water was applied in all tested species in all the experiments. In the laboratory, wheat and cowpeas recorded a decrease of germination of 55.3 % and 55.65 % at 8% of aqueous thorn apple, respectively. The lowest germination percentage was recorded where 8% concentration of *D. stramonium* was applied on wheat and cowpeas across all the three environments.

Radicle and root length

The results indicated that as the concentration of thorn apple aqueous extract was increased from 0 to 8%, this led to a decrease in root length in all tested species across all the environments. Results from the laboratory and field showed highly significant reductions ($p<0.001$) effects of *D. stramonium* on root length across all the concentrations for both crops as shown in Table 1.

Plumule and shoot length

Results from the study showed that the plumule and shoot length of both tested species were reduced significantly ($p<0.001$) by aqueous leaf extracts of *D. stramonium* concentrations across all the environments shown in Table 2.

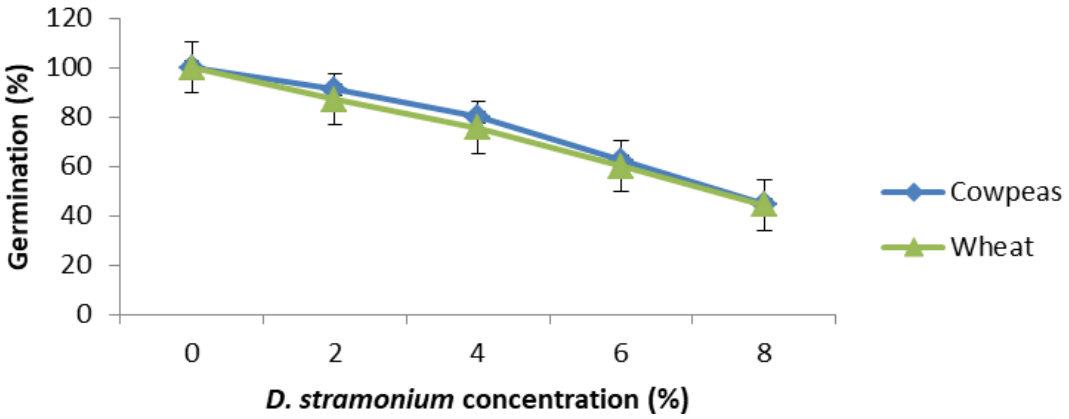

Figure 1. Effects of aqueous concentration of thorn apple on seed germination of crops in the laboratory

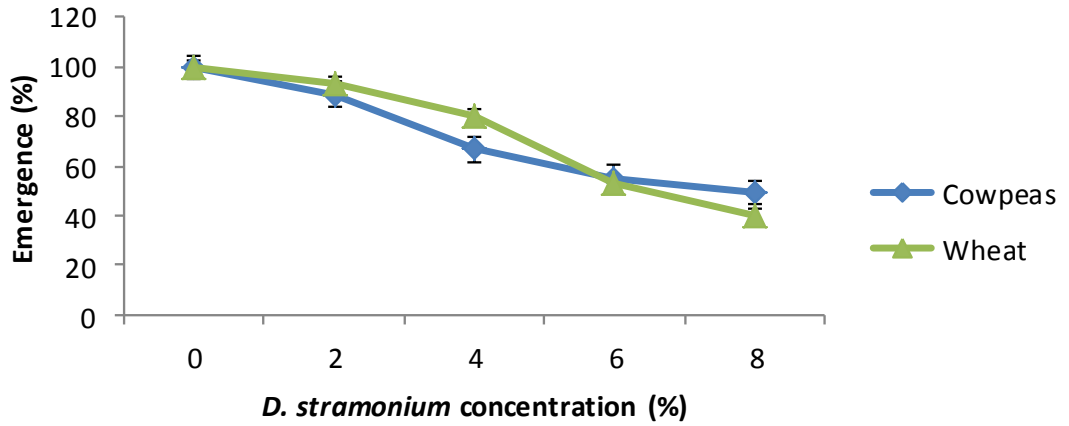

Figure 2. Effects of aqueous concentrations of thorn apple on seed emergence of crops in the field.

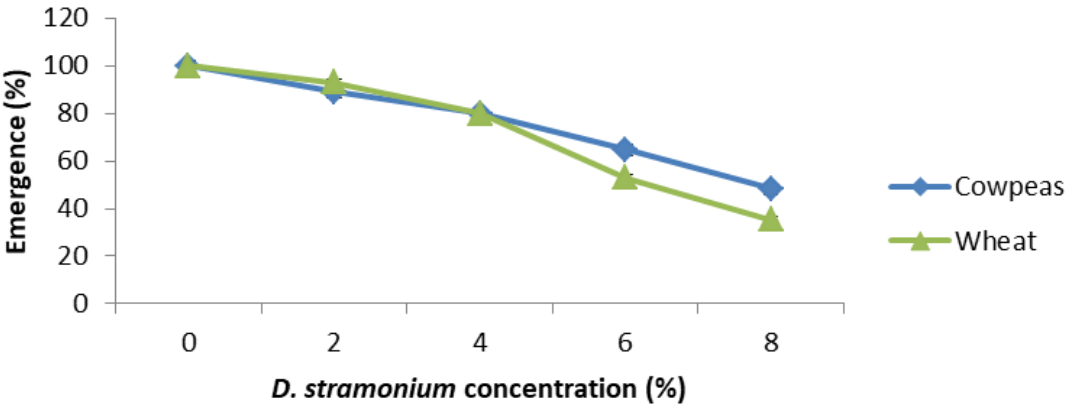

Figure 3. Effects of thorn apple aqueous concentrations on seed emergence of crops in the greenhouse.

Seedling fresh weight

Results indicated that the effect of the *D. stramonium* aqueous extracts was concentration dependent; it significantly ($p<0.001$) reduced seedling fresh biomass shown in Table 3. The highest reduction was recorded at

Table 1. Effects of aqueous concentrations of thorn apple on radical length of tested crops in the laboratory and field.

Concentration (%)	Laboratory		Field		Green house	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	51.03[a]	43.23[a]	65.23[a]	74.70[a]	65.03[a]	74.90[a]
2	42.23[b]	41.17[b]	62.93[b]	69.53a[b]	62.87[b]	68.97[b]
4	43.1[c]	37.33[c]	58.87[c]	66.50[bc]	58.83[c]	65.07[c]
6	40.1[d]	35.13[d]	54.23[d]	61.70[c]	53.47[d]	59.43[d]
8	38.43[e]	34.10[e]	51.03	55.37[d]	50.90[e]	51.67[e]
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	0.8	0.2	1.7	4.5	2	0.7

*Means followed by the same letter in the same column are not significantly different.

Table 2. Effects of different aqueous concentrations on early seedling growth (plumule length) in the laboratory, field and greenhouse.

Concentration (%)	Laboratory		Field		Green house	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	21.38[a]	43.23[a]	79.03[a]	85.60[a]	79.10[a]	85.73[a]
2	19.78[b]	41.17[b]	76.87[ab]	83.80[b]	76.90[ab]	83.67[b]
4	18.12[c]	37.33[c]	75.10[b]	79.03[c]	74.80[b]	78.93[c]
6	17.12[d]	35.13[d]	69.57[c]	76.20[d]	69.83[c]	75.90[d]
8	16.40[e]	34.10[e]	66.50[d]	70.60[e]	66.67[d]	70.40[e]
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	3.2	0.2	1.6	0.5	1.8	0.7

*Means followed by the same letter in the same column are not significantly different.

Table 3. Effects of different aqueous concentrations of thorn apple on fresh weight of crops in the greenhouse and field.

Concentration (%)	Greenhouse		Field	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	2.537[a]	5.527[a]	2.510[a]	5.497[a]
2	2.407[b]	5.390[b]	2.370[b]	5.370[b]
4	2.273[c]	5.237[c]	2.217[c]	5.30[c]
6	2.177[d]	5.060[d]	2.160[d]	5.047[d]
8	2.017[e]	4.957[e]	2.017[e]	4.947[e]
P value	<0.001	<0.001	<0.001	<0.001
CV (%)	0.5	0.6	1.1	0.5

*Means followed by the same letter in the same column are not significantly different.

8% concentration in both field and greenhouse environments.

Dry weight

From the study, results indicated that as the concentration of thorn apple, aqueous extract increased from 0 to 8%; seedling dry weight was significantly ($p<0.001$) reduced in both field and greenhouse environments shown in Table 4.

DISCUSSION

Results in this study indicated that where distilled water

Table 4. Effects of different aqueous concentrations of thorn apple on dry weight of crops in the field and greenhouse.

Concentration (%)	Greenhouse		Field	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	1.0867[a]	2.277[a]	1.500[a]	2.230[a]
2	0.9233[b]	2.140[b]	0.9100[b]	2.103[b]
4	0.823[c]	1.987[c]	0.7567[c]	1.963[c]
6	0.7267[d]	1.810[d]	0.700[c]	1.780[d]
8	0.5667[e]	1.707e	0.5567[d]	1.680[e]
P value	<0.001	<0.001	<0.001	<0.001
CV (%)	3.7	1.5	4.2	4.6

*Means followed by the same letter in the same column are not significantly different.

was applied germination was very high due to lack of germination inhibition. This shows that water is necessary for germination to take place; it triggers biochemical reactions. Seed germination on all tested species might have been inhibited by the presence of aqueous thorn apple extracts; these results were in line with those by Hassannejad and Ghafarbi (2013). This could be attributed to reduced water uptake by the seeds in the presence of allelochemicals found in thorn apple (EL-shora et al., 2015a). According to Oyun (2006), allelochemicals inhibit water absorption which is a precursor to physiological processes (hydrolysis of food nutrients) that should occur in seed before germination is triggered hence affecting germination (Altikat et al., 2013; Ullah et al., 2015). Reduced water uptake resulted in reduced imbibitions leading to delayed germination and emergence. Results from the field and greenhouse experiments showed the same trend as compared to the laboratory; however, seed emergence was not affected at large. In the laboratory, seed were always exposed directly to aqueous extracts which might have caused significant effects as compared to the pots which did have soil. When a comparison is made in terms of seeds that germinated in petri dishes and pots results showed that seeds germinated better in pots as compared to petri dishes. Allelochemicals present in *D. stramonium* might have been inactivated in the soil by several factors such as complexation with soil colloids, chelation with ions and decomposition by micro-organisms. In an experiment by Wasim et al. (2008) on effects of allelochemicals on vigour of *T. aestivum* (variety GW273), results showed that allelochemicals are known to disturb the activity of enzymes that break down starch that nourish the growing embryo during germination. Results obtained were also in agreement to with those by Alam and Islam (2002), Altikat et al. (2013), and Ullah et al. (2015) who found that allelochemicals disturb activities of peroxidase alpha-amylase and acid phosphates which aid the breaking down of starch in wheat. From the study results observed, they were in agreement with those obtained by EL-Shora and Abd EL-Gawad (2014), EL-Shora et al., (2015a) and Singh et al. (1992) who reported that biological activities of receiver plants to allelochemicals are known to be concentration dependent with inhibition increasing as the concentration of *D. stramonium* increased from 0 to 8%.

Results on root length from the laboratory showed highly significant effects of *D. stramonium* on root length on both crops. This might have been promoted by similar physiological processes that occur during cell division, cell expansion and auxin production. When results of root length in petri dishes and pots were compared, they showed that seedlings in pots had greater root length than those in petri dishes. From the study, this trend might have been brought by continuous exposure of roots directly to free aqueous *D. stramonium* in the laboratory which might have reduced root length. In pot experiments, however, allelochemicals present in *D. stramonium* aqueous extract might have been inactivated in the soil thereby significantly reducing root length to a higher extent. From this study, results indicated that plant roots exposed to *D. stramonium* became brownish and shorter in length. The results were in agreement with those by Butnariu (2012) who concluded that *D. stramonium* contains a series of alkaloids such as atropine (d-1-hiosciamine), hiosciamine, and scopolamine which inhibit the growth and development of roots. The allelochemicals present in *D. stramonium* might have interfered with auxin production on root tips which promotes root elongation and expansion (Gholami et al., 2011). Allelochemicals from thorn apple in this study exhibited allelopathic potential on seedling growth of tested species. From the study, results indicated that these extracts might have some inhibitory alkaloids which might have reduced seedling growth of the tested species by hindering cell division, development and cell elongation on root tips. Results from the study showed that seedling growth (radical and root length) was reduced and plant roots exposed to allelochemicals became brownish. This might have been the mode of action of allelochemicals which interfered with hormone production on root tips. Cowpea and wheat became shorter and showed signs of necrosis when they were exposed to *D. stramonium* aqueous extract. The results

were in agreement with those by Lorber and Muller (1976) who observed that allelochemicals caused structural damage to root tips of target plants leading to reduced root size and length. From the study, these allelochemicals might have interfered with hormones that encourage growth processes, development, cell elongation, cell division and cell enlargement on younger active root tips where mitosis is active (EL-Shora et al., 2015a). These findings were similar to those by Gholami et al. (2011) who concluded that alkaloids present in *D. stramonium* (hiosciamine and scopolamine) can reduce cell division and auxin synthesis that induces the growth of shoots and roots.

Results from the study showed that the plumule and shoot length of both tested species was reduced by aqueous leaf extracts of *D. stramonium.* The results obtained suggest that the reduction in shoot length might be attributed to the presence of *D. stramonium* aqueous extracts. Reduction in plumule length and shoot length might be due to the presence of allelochemicals in *D. stramonium* which might have inhibited mitosis. The presence of allelochemicals might have caused disassembling of microtubules leading to shortened spindle fibres essential for cell division to take place. *D. stramonium* aqueous extract might have inhibited protein synthesis in shoot meristems in tested species leading to reduced shoot length. The results corroborate with those by Hussain and Reigosa (2011) who observed the inhibitory effects of allelochemicals (phenolic compounds) on root and shoot length of *Dactylusglomerata*, *Loliumperenne* and *Rumexacetosa*. The allelopathic effects of *D. stramonium* on shoot growth of tested species increased as their concentration was increasing.

Results in the greenhouse and field experiments indicated that the effect of the *D. stramonium* aqueous extracts on cowpeas and wheat was concentration dependent (EL-Shora and Abd EL-Gawad, 2014; EL-Shora et al., 2015a). The presence of *D. stramonium* aqueous extracts might have interfered with photosynthesis leading to drastic changes in the physiology of plants. Reduction in photosynthesis might have led to reduced plant growth and reduced fresh biomass accumulation due to reduced water content in the tested species (EL-Shora et al., 2018). According to Oyun (2006), allelochemicals inhibit water absorption which is a precursor to physiological processes like photosynthesis. Reduced water uptake might have promoted reduced fresh biomass accumulation on tested species. Photosynthesis encourages vegetative growth and rapid accumulation of fresh biomass. The effects of allelopathy on seedling fresh weight and growth of plants may occur through a variety of mechanisms including reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced water uptake, inhibited photosynthesis, respiration, inhibited protein formation and decreased permeability of cell membranes and/or inhibition of enzyme action (Rice, 1984).

Reduction in total biomass dry matter correlated with root and shoot length; this is in line with a study by Garcia et al. (2002) who observed that reduction in biomass might be due to stunted and reduced seedling growth.

Conclusion

It can be concluded from the results that allelochemicals present in the aqueous extract of *D. stramonium* suppressed germination and early seedling growth of *Triticum aestivum* and *Vigna unguiculata.* Sensitivity of the tested species differed; the seedling growth of the tested species (root length) was more suppressed than the germination. Wheat and cowpeas are susceptible to *D. stramonium* allelochemicals hence cannot be used as bio herbicides in these two arable crops. However, further research is necessary for comparison of more than one season in different agro-ecological zones. The results showed that *D. stramonium* has allelopathic effects on wheat and cowpeas, hence cannot be used as a bio herbicide since it is non selective to the crops studied. There is therefore need for further research on screening of arable crops against the allelopathic effects of *D. Stramonium.* This will help to identify arable crops which are not negatively affected by allelochemicals from *D. stramonium* weed, so that it can be used as a selective bio herbicide against other weeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Special thanks go to Midlands State University for the provision of experimental site and apparatus for carrying out the research.

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Vol. 13(29), pp. 1468-1475, 19 July, 2018
DOI: 10.5897/AJAR2018.13118
Article Number: DBF71CF57846
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

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African Journal of Agricultural Research

Full Length Research Paper

Analysis of coffee quality along the coffee value chain in Jimma zone, Ethiopia

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Received 13 March, 2018; Accepted 30 April, 2018

This study assesses the effect of cooperative, certification, private trader, farmers, sorting and processing methods on Arabica coffee quality. Coffee samples were collected from certified cooperatives, non-certified cooperatives, private traders and farmers (members of certified cooperatives, non-certified cooperatives and non-members of cooperatives). The study showed that coffee beans sampled from cooperatives had higher quality scores and were classified as specialty 1 (Q1) (33%) or specialty 2 (Q2) (67%). About 78% of coffee beans sampled from private traders fall in grade 3, while 22% of their beans qualified for Q2. Coffee certification, in general, did not add any value to coffee quality. No quality differences were also observed between coffee beans sampled from farmers. Coffee quality differences were observed between coffee processing methods. Dry processing method improved coffee quality. However, this can only be achieved by using ripe red cherries. Cherry sorting also improved coffee quality and the percentage of coffee samples that fall in Q1. In general, proper coffee cherries type together with site specific coffee processing approach helps coffee actors to produce high quality coffee.

Key words: Arabica coffee, flavor, body, specialty, cherry, acidity.

INTRODUCTION

Coffee is the world's favorite beverage and most traded commodity (Barbosa et al., 2014; Davis et al., 2012; Murthy and Naidu, 2012). Coffee quality is an important attribute in the international market and triggers coffee producing countries to produce high quality coffees (Curzi et al., 2014). Ethiopia is known for the origin and wide diversity of Arabica coffee and has enormous, unexplored potential to produce top specialty coffees (Anthony et al., 2001; Coste et al., 1992). Coffee production is the backbone of the Ethiopian economy, contributing 25 to 30% of total export earnings (Tefera and Tefera, 2014). Coffee further plays a major role in sustaining the livelihoods of more than 15 million households in the country (Davis et al., 2012). Majority

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of coffee produced in the country is grown by smallholders farmers, while about 5% comes from large coffee plantations (Tefera, 2015).

Coffee produced by these smallholder farmers reaches consumers by passing through different value chain actors. In Ethiopia, coffee value chain actors include: input providers, producers (smallholder farmers, private growers and coffee plantation), private trader, cooperatives, unions (association of cooperatives), Ethiopia Commodity Exchange (ECX), various government institutions, exporters and finally the consumer (Gemech and Struthers, 2007). Cooperatives enhance the competitiveness of the smallholder coffee farmers through modernization of coffee production and marketing system (Bernard and Spielman, 2009; Dorsey and Assefa, 2005). Cooperatives also enable farmers to improve coffee quality and ultimately their income (Dempsey and Campbell, 2006; Kodama, 2007).

Unions are associations of cooperatives that enhance the economic scale by increasing the bargaining power of cooperatives in selling their product (Emana, 2009; Meijerink et al., 2010). Unions took the lead to establish international market linkage and facilitate direct export on behalf of smallholder coffee producers. This linkage encourages coffee growers to produce quality product and receive premium prices (Dempsey, 2006). Hence, the “new” coffee value chain: cooperatives and unions, have gained particular importance on international specialty coffee market (Stellmacher, 2011) and increased local farmers’ market share in the international market (Emana, 2009; Kodama, 2007).

In consumer countries, in addition to quality, there is also a growing demand for healthier and eco-friendly produced coffee (Giovannucci et al., 2014; Jena et al., 2012; Stellmacher, 2007). For differentiation and creation of niche markets for such coffee products, certification has become an important tool (Grote et al., 2007; Daniele, 2008). In northern Nicaragua, smallholder coffee producers and certified cooperatives significantly benefited from certification (Bacon, 2005; Dorr et al., 2010; Philpott et al., 2007; Poncelet et al., 2005). In Ethiopia, however, there are no studies that report impacts of cooperatives and certification on coffee bean quality.

Postharvest processing method is another key factor that influences the final coffee quality and chemical compounds (Bytof et al., 2005; Duarte et al., 2010; Knopp et al., 2006; Selmar et al., 2002). In Ethiopia, about 70 to 80% coffee beans are processed via dry processing methods while the remaining 20 to 30% are washed processed coffee beans (Bart et al., 2014). Washed processing methods use red ripe cherries while cherries of different ripening stages are usually processed via dry processing method. This might affect qualities of dry processed coffee beans. Almost all cooperatives use washed processing. This method is, however, expensive, consume large amounts of water and pollute the environment. Hence, this needs further research to show the impacts of processing methods on coffee quality by considering identical coffee cherries. This study therefore, aimed to determine the effects of certification, processing methods, coffee bean sorting and actors in the value chain on coffee bean quality.

MATERIALS AND METHODS

Study site

The experiment was carried out in three districts (Mana, Goma and Limu) of Jimma zone, Oromia regional state, Ethiopia. Mana district is located at an elevation of 1400 - 2610 m asl and 7° 67' N, 37° 07' E with mean annual temperature and rainfall of 20.5°C and 1525 mm, respectively. The soil of the area is characterized as a Nitisol, with pH ranging from 4.5 to 5.5 (ARDO, 2008). Goma district is located at an elevation of 1400 - 2270 m asl and geographical location of 7° 57' N, 36° 42' E, with annual mean temperature and rainfall of 21.7°C and 1600 mm, respectively. The soil of the area is characterized as an Eutric Nitisol, with pH ranging from 4.5 to 6.0 (IPMS, 2007). The third site, Limu, is located at an elevation of 1600 - 1800 m asl, 8° 05' N, 36° 57' E, with an annual mean temperature and rainfall of 20.4°C and 1616 mm, respectively. The soils are dominated by Eutric Nitisols with pH ranging of 4.5 to 5.8.

Treatments and experimental design

For this study, coffee actors along the value chain (cooperatives, private traders and farmers) of three districts (Mana, Goma and Limu) were considered. The study was composed of three experiments. In experiment 1, washed processed coffee beans were sampled from cooperatives: certified cooperatives (CC) and non-certified cooperatives (NCC) and private traders (PT). In experiment 2, red coffee cherry samples were collected from farmers: members of certified cooperatives (FMCC), members of non-certified cooperatives (FMNCC) and non-members of cooperatives (FNMC). In experiment 3, red coffee cherry samples were also collected from farmers; members of certified cooperatives (FMCC), members of non-certified cooperatives (FMNCC) and non-members of cooperatives (FNMC). The collected samples were sorted out as ripe and unripe cherries and were then subjected to dry and wash processing methods. In all the experiments, coffee samples were collected from October 2012 to February 2013. Detailed description of each experiment is presented as follows.

Experiment 1

Six coffee cooperatives (three certified and three non-certified) were selected from both Mana and Goma district. In Limu, since all cooperatives were certified, three cooperatives were randomly selected. In addition, nine private traders, three from each district, were randomly selected. Certified and non-certified cooperatives bought coffee cherries from their own respective members while private traders bought from farmers who were not members of cooperatives. For each treatment, composite samples of one kilogram of washed processed green coffee beans were collected for quality analysis. These composite samples were taken from 30 bags containing green coffee beans. The experiment was arranged in a “split plot” design, with district as “main plot” and three levels of actors (certified cooperatives, non certified cooperatives and private traders) as “sub-plot”.

Table 1. Effect of actors (A): certified cooperatives (CC), non-certified cooperatives (NCC) and private traders (PT) on preliminary cup quality (PCQ), preliminary total quality (PTQ), total specialty cup quality (TSCQ) and specific specialty cup quality scores of washed processed coffee beans.

Factors		PCQ	PTQ	TSCQ	Specific specialty cup quality attributes					
					OCP[1]	Acidity	Body	Aroma	Flavour	AT[2]
A	CC	47.3 ± 0.6[b]	74.3 ± 1.0[b]	82.3 ± 0.5[a]	7.5 ± 0.2[b]	7.7 ± 0.1[a]	7.4 ± 0.1[a]	7.4 ±<0.1[a]	7.4 ± 0.1[a]	7.3 ± 0.1[a]
	NCC	50.0 ± 0.6[a]	77.7 ± 1.1[a]	84.3 ± 0.9[a]	7.9 ± 0.2[a]	8.1 ± 0.2[a]	7.6 ± 0.2[a]	7.7 ± 0.1[a]	7.8 ± 0.2[a]	7.5 ± 0.2[a]
	PT	46.8 ± 0.5[b]	73.6 ± 0.9[b]	78.8 ± 0.9[b]	6.9 ± 0.2[b]	7.2 ± 0.1[b]	6.9 ± 0.1[b]	6.9 ±<0.1[b]	7.1 ± 0.1[b]	6.9 ± 0.1[b]
***P*- value**		**0.0017**	**0.009**	**0.0036**	**0.027**	**0.03**	**0.011**	**0.003**	**0.013**	**0.013**

[1]Overall cup preference, [2]After taste, different letters in the same column indicate significant difference, according to Tukey's HSD post hoc test (P<0.01) are shown as mean ± standard error.

Experiment 2

The aim of this experiment is to examine the variability of coffee quality among farmers (members of certified cooperatives, members of non-certified cooperatives and non-member of cooperatives) and also processing method (dry and washed). From each three districts, six farmers were randomly selected and coffee cherries were collected from local market places where farmers sell cherries to either cooperatives or private traders. The collected samples (14 kg from each farmer) were subjected to two processing methods (dry and washed) at Goma I and Limu washing stations. These coffee samples were then processed without sorting out unripe, ripe and overripe cherries. The experiment was arranged in a "split-split plot" design with districts as "main plot", farmer type as "sub-plot" and processing type as "sub-sub-plot".

Experiment 3

In this experiment, coffee cherries collected from farmers (members and non-members of cooperatives) were sorted for ripe, unripe and overripe coffee cherries before processing. For this, only Goma district was considered and coffee cherries (28 kg per farmer) were collected at the local market from six farmers per type. Half of the cherries (14 kg) were sorted as ripe, unripe and overripe cherries and only clean and ripe coffee cherries were subjected to dry and wash processing. The other 14 kg of unsorted coffee cherries were also subjected to dry and wash processing methods. The experiment was arranged in a "split-split plot" design with actors as "main plot", sorting treatment as "sub-plot" and processing methods (washed and dry processing) as "sub-sub-plot".

Coffee quality analysis

Coffee quality was assessed based on both physical and cup quality analysis. Cup tasting was performed by a team of three experts working in Ethiopia Commodity Exchange (Tolessa et al., 2016). For scores higher than 70 cup quality, specialty coffees were further assessed for overall cup preference, acidity, body, aroma, flavour, aftertaste, uniformity, cup cleanness, sweetness and balance.

Data analysis

Data were analyzed using SAS (v. 9.2, SAS Institute Inc., Cary, NC USA) mixed model procedure for a split plot design. Significant differences between treatment means were determined using Tukey's honest significant difference (HSD) test at $P < 0.01$ and $P < 0.05$.

RESULTS

Coffee quality

The result of experiment 1 (Table 1) indicated that different coffee actors significantly influenced ($P < 0.01$) preliminary cup quality, preliminary total quality, total specialty cup quality, acidity and aroma of coffee beans. Coffee beans of non-certified cooperatives had higher quality scores for preliminary cup quality (50.0) and preliminary total quality (77.7) than beans from certified cooperatives and private traders (Table 1). Total specialty cup quality of certified (82.3) and non-certified cooperatives (84.3) was higher as compared to private trader's coffee (78.8). Acidity and aroma scores were also higher for certified (7.7 and 7.4) and non-certified (8.1 and 7.7) cooperatives as compared to private traders (7.2 and 6.9), respectively (Table 1). Different coffee growing districts, on the other hand, did not show any significant effect on all coffee quality attributes. In experiment 2 (Table 2), interactions between actors and processing methods significantly affected overall cup preference ($P < 0.01$). Dry processed coffee beans sampled from non-certified cooperatives gave the highest quality scores as compared to beans from any other treatment combinations (Table 3).

Districts as main effect had a significant effect on preliminary cup quality, total specialty cup quality and flavor characteristics of coffee bean (Table 3). For these quality attributes, coffee beans sampled from Limu had the highest scores while coffees from Mana had the lowest. Similarly, dry processed coffee beans had higher physical

Table 2. The interactive effect of actors (A): farmers who are a member of certified cooperatives (FMCC), farmers who are a member of non- certified cooperatives (FMNCC), farmers who are non-member of cooperatives (FNMC) and processing methods (PM): dry (DP) and washed (W) method on total specialty cup quality (TSCQ) and specific specialty cup quality scores.

A	PM	TSCQ	Specific specialty cup quality attributes					
			OCP[1]	Acidity	Body	Aroma	Flavour	AT[2]
FMCC	DP	84.2 ± 0.3[a]	7.8 ± 0.1[a]	7.9 ± <0.1[a]	7.9 ± 0.1[a]	7.6 ±<0.1[a]	7.7± 0.1[a]	7.7 ±<0.1[a]
	W	82.8 ± 0.4[b]	7.5 ± 0.1[b]	7.7 ± 0.1[b]	7.5 ± 0.1[a]	7.5 ±<0.1[a]	7.6 ± 0.1[a]	7.4 ± 0.1[a]
FMNCC	DP	84.7 ± 0.3[a]	7.9 ± 0.1[a]	7.9 ± 0.1[a]	7.9 ± 0.1[a]	7.7 ± 0.1[a]	7.8 ± 0.1[a]	7.8 ±<0.1[a]
	W	82.9 ± 0.6[b]	7.7 ± 0.1[ab]	7.8 ± 0.1[b]	7.5 ± 0.1[a]	7.6 ± 0.1[a]	7.7 ±0.1[a]	7.6 ± 0.1[a]
FNMC	DP	83.5 ± 0.3[a]	7.6 ±<0.1[ab]	7.9 ± <0.1[ab]	7.8± <0.1[a]	7.5 ±<0.1	7.7 ±<0.1[a]	7.6 ±<0.1[a]
	W	83.3 ± 0.2[a]	7.7 ± 0.1[ab]	7.8 ± <0.1[ab]	7.6 ±<0.1[a]	7.5 ±<0.1[a]	7.6 ±<0.1[a]	7.5 ±<0.1[a]
***P* -value**		**0.038**	**0.0062**	**0.039**	**0.115**	**0.787**	**0.76**	**0.32**

[1]Overall cup preference, [2]After taste, different letters in the same column indicate significant difference according to Tukey's HSD post hoc test ($P<0.01$); mean ± standard error.

Table 3. Effect of district (D), actors (A): farmers who are a member of certified cooperatives (FMCC), farmers who are a member of non- certified cooperatives (FMNCC), farmers who are not member of cooperatives (FNMC) and processing methods (PM): dry (DP) and washed (W) methods on physical quality (PQ), preliminary cup quality (PCQ), preliminary total quality (PTQ), total specialty cup quality (TSCQ) and specific specialty cup quality scores.

Factor		PQ	PCQ	PTQ	TSCQ	Specific specialty cup quality attributes					
						OCP[1]	Acidity	Body	Aroma	Flavour	AT[1]
D	Mana	35.1 ± 0.7[a]	47.5 ± 0.8[b]	82.6 ± 0.4[a]	82.8 ± 0.3[b]	7.6 ±<0.1[b]	7.8 ± 0.1[a]	7.6 ± 0.1[b]	7.5 ±<0.1[a]	7.6 ±<0.1[b]	7.5 ±<0.1[b]
	Goma	34.2 ± 0.7[a]	48.9 ± 0.9[ab]	83.6 ± 0.5[a]	83.8 ± 0.3[ab]	7.8 ± 0.1[a]	7.9 ±<0.1[a]	7.8 ±<0.1[a]	7.5 ±<0.1[a]	7.7 ±<0.1[ab]	7.6 ±<0.1[ab]
	Limu	34.3 ±1.2[a]	49.6 ± 1.4[a]	83.9 ± 0.5[a]	84.1 ± 0.2[a]	7.8 ±<0.1[a]	7.9 ±<0.1[a]	7.7 ±< 0.1[a]	7.6 ±<0.1[a]	7.8 ± 0.1[a]	7.9 ±<0.1[a]
	***P* -value**	**0.841**	**0.0072**	**0.466**	**0.008**	**0.014**	**0.058**	**0.014**	**0.106**	**0.006**	**0.031**
A	FMCC	35.0 **±** 0.8[a]	48.3 ± 0.9[b]	83.4 ± 0.4[a]	83.5 ± 0.3[a]	7.7 ± 0.1[a]	7.8 ±<0.1[a]	7.7 ±<0.1[a]	7.5 ±<0.1[a]	7.7 ± 0.1[a]	7.6 ±<0.1[a]
	FMNCC	35.0 ± 0.7[a]	49.5 ± 0.8[a]	84.5 ± 0.5[a]	83.8 ± 0.3[a]	7.8 ± 0.1[a]	7.9 ± 0.1[a]	7. 7± 0.1[a]	7.6 ±<0.1[a]	7.8 ± 0.1[a]	7.7 ±<0.1[a]
	FNMC	34.3 ± 0.8[a]	48.2 ± 0.9[b]	82.5 ±0.5[a]	83.4 ± 0.2[a]	7.7 ±<0.1[a]	7.8 ± <0.1[a]	7.7 ±<0.1[a]	7.5 ±<0.1[a]	7.7 ± <0.1[a]	7.6 ±<0.1[a]
	***P* -value**	**0.831**	**0.04**	**0.301**	**0.38**	**0.31**	**0.768**	**0.94**	**0.0.06**	**0.31**	**0.24**
PM	DP	37.1 ± 0.5[a]	48.8 ± 0.7[a]	85.9 ± 0.4[a]	84.1 ± 0.2[a]	7.8 ±<0.1[a]	7.9 ±<0.1[a]	7.8 ±<0.1[a]	7.9 ±<0.1[a]	7.7 ± <0.1[a]	7.7 ±<0.1[a]
	W	32.5 ± 0.6[b]	48.3 ± 0.8[a]	80.8 ± 0.4[b]	82.9 ± 0.2[b]	7.5 ± 0.1[b]	7.8 ±<0.1[b]	7.6± <0.1[b]	7.5 ±<0.1[b]	7.5 ± <0.1[b]	7.4 ±<0.1[b]
	***P* -value**	**<0.0001**	**0.22**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.008**	**0.01**	**<0.001**

[1]Overall cup preference, [2]After taste, different letters in the same column indicate significant difference, according to Tukey's HSD post hoc test ($P<0.01$); results are shown as mean ± standard error.

quality, preliminary total quality, total specialty cup quality and specialty cup quality scores than washed processed coffee beans. Preliminary cup

Table 4. Effect of actors (A): farmers who are members of certified cooperatives (FMCC), farmers who are members of non-certified cooperatives (FMNCC), farmers who are non-members of cooperatives (FNMC), sorting treatment (Tr): sorted and unsorted and processing methods (PM): dry (DP) and washed (W) methods on physical quality (PQ), preliminary total quality (PTQ), total specialty cup quality (TSCQ) and specific specialty cup quality scores.

	Factor	PQ	PTQ	TSCQ	Specific specialty cup quality attributes				
					OCP 1	Acidity	Body	Flavour	AT2
A	FMCC	38.2 ± 1.2[a]	83.0 ± 0.9[a]	83.8 ± 0.7[a]	7.7 ± 0.1[a]	7.7 ± 0.1[a]	7.7 ± 0.1[a]	7.6 ± 0.1[a]	7.6 ± 0.1[a]
	FMNCC	37.3 ± 0.9[a]	84.1 ± 0.6[a]	84.6 ± 0.4[a]	7.8 ± 0.1[a]	7.9 ± 0.1[a]	7.8 ± 0.1[a]	7.8 ± 0.1[a]	7.6 ± 0.1[a]
	FNMC	38.2 ±0.5 [a]	82.5 ± 0.5[a]	83.9 ± 0.5[a]	7.6 ± 0.1[a]	7.8 ± 0.1[a]	7.7 ± 0.1[a]	7.7 ± 0.1[a]	7.5 ± 0.1[a]
	***P* -value**	**0.03**	**0.025**	**0.1**	**0.13**	**0.03**	**0.2**	**0.09**	**0.49**
Tr	sorted	38.3 ± 0.7[a]	85.3 ± 0.6[a]	85.2 ± 0.3[a]	7.9 ± 0.1[a]	7.9 ± 0.1[a]	7.9 ± 0.1[a]	7.8 ± 0.1[a]	7.7 ± 0.1[a]
	Unsorted	36.5 ± 0.6[b]	80.0 ± 0.4[b]	83.0 ± 0.4 [b]	7.5 ± 0.1[b]	7.7 ± 0.1[b]	7.5 ± 0.1[b]	7.6 ± 0.1[b]	7.4 ± 0.1[b]
	P-value	**0.004**	**0.0004**	**<0.0001**	**0.001**	**0.002**	**0.003**	**0.0028**	**0.003**
PM	DP	38.9 ± 0.8[a]	85.1 ± 0.6[a]	84.8 ± 0.4[a]	7.9 ± 0.1[a]	7.9 ± 0.1[a]	7.9 ± 0.1[a]	7.8 ± 0.1[a]	7.7 ± 0.1[a]
	W	35.9 ± 0.7[a]	81.2 ± 0.5[b]	83.4 ± 0.4[b]	7.6 ± 0.1[b]	7.7 ± 0.1[b]	7.6 ± 0.1[a]	7.6 ± 0.1[a]	7.5 ± 0.1[a]
	***P* -value**	**0.009**	**0.0003**	**0.0009**	**<0.001**	**0.002**	**0.04**	**0.02**	**0.01**

Overall cup preference, [2]After taste, different letters in the same column indicate significant difference, according to Tukey's HSD post hoc test (P<0.01); results are shown as mean ± standard error; results are shown as mean ± standard error

Table 5. Percentage of coffee samples under specialty 1 (Q1), specialty 2 (Q2) and grade 3.

Actors[1]	Q1	Q2	Grade 3
CC	33	67	0
NCC	50	50	0
PT	0	22	78

[1]Coffee beans were obtained from different actors: certified cooperatives (CC), non-certified cooperatives (NCC) and private traders (PT).

quality, on the other hand, was not significantly affected by processing methods (P > 0.01; Table 3). In addition, no significant effect of actors was observed for all bean quality attributes (P > 0.01; Table 3).

The result of experiment 3 (Table 5) showed that cherry sorting significantly affected (P < 0.01) all coffee quality attributes except preliminary cup quality. As compared to the unsorted coffee beans, sorted coffee beans gave higher quality scores for physical quality (38.3), preliminary total quality (85.3) total specialty cup quality (85.2), overall cup preference (7.9), acidity (7.9), body (7.9), aroma (7.8), flavour (7.8) and aftertaste (7.7) (Table 4).

Coffee quality attributes were also significantly influenced by processing method (P < 0.01). Dry processing methods enhanced quality scores of physical quality (38.9), preliminary total quality (85.1), total specialty cup quality (84.8), overall cup preference (7.9) and acidity (7.9). Processing methods, on the other hand, did not significantly affect preliminary cup quality, body, aroma, flavour and aftertaste (P > 0.01). Differences among farmers on the other hand, did not significantly affect (P > 0.01; Table 4) coffee quality. Generally, the three and two - way interactions between farmers, sorting treatments and processing methods did not significantly affect coffee quality attributes.

DISCUSSION

This study showed that coffee bean quality attributes: preliminary cup quality, preliminary total quality, total specialty cup quality, acidity and aroma showed significant differences among the different coffee actors in the value chain. Coffee beans that passed through a cooperative system had higher quality scores than beans that passed via private traders. An unexpected result from this study was that coffee certification did not result in any quality improvement. This is probably due to the fact that certification mainly focuses on promotion of socioeconomic advantage and environmental sustainability rather than coffee quality. In other studies by Bart et al. (2014), it was reported that certification did not necessarily affect coffee quality but marketability and prices. The certification schemes should therefore include criteria that improve farmer's management practice and their livelihood. The certification cost, on the other hand, is very high (ca. $6000) and probably restricts the practicability of the scheme.

Coffee beans collected from private traders were classified mainly into commercial grade 3 (ca. 78%), only 22% of the samples were qualified as Q2 and none as Q1 (Table 5). Quality differences between cooperative and private traders coffee beans could be explained by the differences in type of coffee cherries (red ripe, unripe and overripe) delivered by farmers and growing environment. In coffee cooperatives, coffee cherries

Table 6. Percentage of coffee samples under specialty 1 (Q1), specialty 2 (Q2) and grade 3 obtained from different actors: farmers, member of certified cooperatives (FMCC), farmers, member of non-certified cooperatives (FMNCC), farmers, non-member of cooperatives (FNMC) and processed by dry and washed methods.

Actors	PM	Q1	Q2	Grade 3
FMCC	DP	33	61	6
	W	16	78	6
FMNCC	DP	42	50	8
	W	25	67	8
FNMC	DP	31	63	6
	W	15	79	6

harvested from different production sites are less likely mixed with each other and are processed separately, while private traders collected coffee cherries from different growing environments (altitude) and processed bulk coffee beans regardless of coffee production sites. Majority of coffee beans processed by cooperatives are qualified for specialty coffees which is not the case for private traders. This shows that a site-by-site processing approach (processing coffee harvested from similar growing environments) contributes to improved bean quality and links bean characteristics with production site.

Coffee cooperatives have their own rules on harvesting and handling of coffee beans. They teach and follow up each farmer on how to harvest and handle coffee cherries. Accordingly, farmers in the cooperatives supply red ripe cherries free from any other foreign materials. Premium prices that are paid by the cooperatives also encourage farmers to give due attention to coffee bean quality. In another study, Kodama (2007) also reported that because of premium prices, farmers pay more attention on coffees supplied to cooperatives than to private traders.

The growing demand for specialty coffee market can therefore be much better exploited by coffee cooperative (Dempsey and Campbell, 2006). This indicates that there is potential for Ethiopian coffee cooperatives to produce high quality coffee beans and increase their share in the international specialty coffee market. Apart from quality improvement, the establishment of cooperatives in the study area also enhanced coffee price paid by private traders. In an area without cooperatives, farmers receive low prices ($ 0.3 per kilogram of red cherries) as compared to areas with cooperatives ($ 0.5 per kilogram of red cherries). But, currently, the share of coffee cooperatives in the study area is small; ca. 15 to 20% as compared to the private traders. Thus, increasing cooperatives' share in coffee export might enhance Ethiopian coffee quality in the international market. This can be achieved through institutional interference to encourage and organize farmers into cooperatives.

Site-specific sample collection and processing also reduce coffee bean quality deterioration. However, this requires appropriate processing method. The results in experiment 2 (Tables 2 and 3) supported this hypothesis. Coffee beans collected from farmers not in the cooperatives had comparable quality as compared to those not in cooperatives (Table 3). About 33, 42 and 31% of coffee samples collected from farmers that are members of certified cooperatives (FMCC), members of non-certified cooperatives (FMNCC) and non-members of cooperatives (FNMC) and processed via dry processing were respectively, classified as Q1 (Table 6).

This finding, on the other hand, is an "apparent paradox" to the result obtained for beans collected from cooperatives and private traders. The possible explanation could be that coffee collected from farmers is site specific or less mixed as compared to coffee beans sampled from private traders. Hence, from these result, it can be concluded that the major cause of quality loss by private traders (experiment 1) is bulking of coffee beans from different environments and processing methods.

The result observed in experiment 2 also revealed that differences in processing methods significantly influenced coffee quality attributes (physical quality, preliminary total quality, total specialty cup quality, overall cup preference and acidity). For these quality attributes, dry processing method gave higher quality scores than washed methods. Furthermore, the largest percentage of coffee samples classified as Q1 were from dry compared to washing processing method (Tables 6 and 7). In Ethiopia, however, dry processed coffee beans are often viewed as an inferior coffee and receive the lowest price. This indicates that improving dry processing methods enhance Ethiopian coffee competitiveness and the share of specialty coffee export. Hence, the present study in general demonstrated that applying consistent quality control methods and via dry processing method increases the percentage of specialty coffee beans. Moreover, appropriate drying materials also need to be considered to achieve superior quality dry processed coffee beans (Abasanbi, 2010; Subedi, 2011; Tsegaye et al., 2014).

In this study, it was also found that sorting coffee cherries before processing improved percentage of Q1 coffee beans from 17 to 61% (Table 7). This also indicates that the presence of small portion of low quality cherries e.g. unripe and overripe in a certain coffee batch can cause a dramatic quality loss. However, sorting after harvest may lead to quantity reduction. For this selective picking of ripe red cherries and consequently arranging more harvesting rounds for cherries enhance both quality and quantity of the coffee beans. Moreover, farmers need to be trained on how inappropriate coffee cherries result in a drastic quality deterioration. Strict assessment of the quality of coffee cherries at the local market, sorting and specific site processing further improved coffee bean

Table 7. Percentage of coffee samples within specialty 1 (Q1) and specialty 2 (Q2) obtained from different actors of Goma district, Jimma zone: farmers who are members of certified cooperatives (FMCC), farmers who are members of non-certified cooperatives (FMNCC), farmers who are not members of cooperatives (FNMC), treated (sorted and unsorted cherries) and processed by dry and washed methods.

		Percentage of specialty 1 and 2		
Treatment	**Actors**	**PM**	**Specialty (Q1)**	**Specialty (Q2)**
Sorted	FMCC	DP	100	0
		WP	33	67
	FMNCC	DP	100	0
		WP	33	67
	FNMC	DP	100	0
		WP	0	100
Mean			61	39
Unsorted	FMCC	DP	0	100
		WP	0	100
	FMNCC	DP	33	67
		WP	0	100
	FNMC	DP	33	67
		WP	33	67
Mean			17	83

qualities.

Conclusions

This study in general showed that coffee beans managed by cooperatives had better quality scores than beans managed by private traders. Coffee certification, on the other hand, did not result in any quality improvement. Coffee beans from farmers, non-members of cooperatives had better quality than coffee beans of private traders. The study also revealed that dry processing method improved coffee bean quality and high percentage of these beans fell under Q1 grades than wash processing methods. However, to enhance coffee quality of dry processing method, proper coffee cherries e.g. ripe red and clean cherries need to be considered. Sorting of unripe and overripe coffee cherries also improved coffee quality and all sorted coffee samples of dry processed coffee beans fell under Q1 coffee. Further studies that consider different seasons and other coffee growing regions can substantiate these findings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the coffee cooperatives, private traders and farmers of Mana, Gomma and Limu Disrticts for granting the permission to collect coffee samples for quality analysis. The authors also thank NUFFIC, Netherlands Organization for International Cooperation in Higher Education (NICHE) for financial support. They also acknowledge the Ethiopia Commodity Exchange (ECX) for their kind cooperation in assessing coffee cup quality.

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Vol. 13(29), pp. 1476-1486, 19 July, 2018
DOI: 10.5897/AJAR2017.12597
Article Number: BD9C6C757848
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Sensor-based algorithms to improve barley nitrogen efficiency in Queensland

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Received 17 July, 2017; Accepted 29 August, 2017

The low efficiency of nitrogen (N) fertilizers impels the innovation of current N management strategies in cereal production. Site specific N management is an emerging field providing novel alternatives to current nutrient management practices through canopy sensing. Barley N use efficiency can be enhanced with GreenSeeker proximal sensors, whose optimal utilization requires algorithms. The design of such algorithms required four N rates (0, 50, 100 and 150 kg N ha^{-1}) and in-season sensing of barley canopy reflectance using a handheld GreenSeeker sensor as well as crop N analysis. The N rates produced enough variability in yields, N uptake and normalized difference vegetation index (NDVI) readings together with strong determination coefficients between in-season NDVI values on one hand and on the other hand in-season N uptake (R^2=0.68, p<0.001), forage yield (R^2=0.84, p<0.001), forage N uptake at harvest (R^2 = 0.65, p<0.001), grain yields (R^2=0.88, p<0.001), and grain N uptake (R^2 = 0.84, p<0.001). These factors enabled the development of in-season N fertilizer algorithms for barley grain and forage production. The built algorithms will enable farmers using GreenSeeker sensors to better manage barley N fertilization with positive outcomes for their financial returns and environmental contamination.

Key words: Barley canopy reflectance, nitrogen fertilizer algorithm, GreenSeeker, N use efficiency, normalized difference vegetation index (NDVI), nitrogen uptake.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a highly valued cereal around the world but especially in Australia. In the 2013 world food commodity ranking, barley was the 12th most important and the 4th major cereal right behind wheat, rice, and maize (Food and Agricultural Organization (FAO), 2016). The 2014 world production was estimated at 144 million tons, with Australia ranked 4th for its 9.1 million tons (FAO, 2016). Approximately 60% of Australian barley grains is exported as malt, food, and feed with the largest being feed barley (Barley Australia, 2016). The Australian barley industry can maintain or improve its relative dominance in terms of productivity

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and environmental sustainability by considering nutrient management strategies that enhance N efficiency. Some of these strategies were grounded on the reflectance properties of crop canopies and their correlations with agronomic quantities such as crop biomass, grain yield, and crop N content. These correlations have been used to develop N fertilizer algorithms for in-season fertilization of cereal crops with the aim of maximizing yield and minimizing N losses.

Various investigations have established the strong relationship between barley canopy reflectance and grain yield. One of the earliest studies on barley yield prediction using canopy reflectance was reported in the early 1980s (Pinter Jr et al., 1981). With a handheld radiometer, daily normalized difference vegetation index (NDVI) values of barley canopy were collected at Feekes growth stage (entire ear out of sheath and flowering commencing) to complete senescence. From these daily NDVI values which is a baseline, the NDVI value of a completely senesced barley canopy was subtracted to derive an index that correlated with barley grain yield. This was an advance over a previously cumbersome method that used cumulated green leaf area index from barley heading until maturity to predict grain yield (Watson et al., 1963). Notwithstanding, because this model relied on reflectance measurement at flowering, its use was restrained in predicting grain yield. In the late 1980s, another study compared reflectance factors of single spectral bands and ratios of spectral bands ranging from 400 to 2300 nm on barley (Kleman and Fagerlund, 1987). Although the Infra-red/red ratio strongly correlated with grain yield in the middle of the season, the correlation was inconsistent afterwards. As an alternative approach to improve this correlation, climatic data were suggested and included as input parameters (Kleman and Fagerlund, 1987). Interestingly, about a decade later, a yield prediction model for winter wheat that included growing degree day (GDD) was developed (Raun et al., 2001).

Another vegetation index reported to correlate with barley grain yield is the transformed chlorophyll absorption in reflectance index (TCARI) (Pettersson and Eckersten, 2007). Reflectance was measured at early stem elongation using a hand-held passive sensor. This indicates that TCARI could be used for early yield prediction in barley. However, this also reveals that NDVI, as a sole vegetation index, has not been extensively investigated on barley at early growth stages. Considering that a number of variable rate technology equipment use NDVI, establishing a relationship between NDVI and barley grain yield at early stages of development under Australian conditions would contribute to enhanced barley N efficiency.

An important application of early canopy sensing correlation with yield and N content is the design of in-season N fertilizer algorithms. The N fertilizer algorithm that seems to stand out among others is the N fertilizer algorithm or NFA (Lukina et al., 2001). It has been refined by several other contributors (Freeman et al., 2003; Raun et al., 2005) and evaluated in terms of higher N efficiency and financial returns on such cereal crops as wheat, corn and rice. The NFA was reported to improve the N use efficiency (NUE) by at least 15 and 9%, respectively in winter wheat and corn (Raun, 2002; Tubana et al., 2008). The starting point toward devising N fertilization optimization algorithm (NFOA) developed an index that predicted potential yield based on in-season NDVI readings.

In winter wheat, the potential yield could be predicted at earlier stages of development as Feekes growth stages 4 and 5 (or tillering) by combining NDVI values with GDD (Raun et al., 2001). The estimated yield (EY) was obtained by adding NDVI values at Feekes growth stages 4 and 5 and dividing them by the cumulative GDD between the two measurements. Later, another yield prediction index, the in-season estimate of grain yield (INSEY), was developed (Lukina et al., 2001). It appeared that grain yield prediction could be improved if the estimated yield was obtained by using the cumulative GDD from sowing to sensing as a divisor. Sensing could be done from Feekes growth stages 4 to 6 and there would not be any significant improvement in the yield prediction regardless of the number of sensing performed within that window. Moreover, yield prediction would be less affected regardless of the NDVI being used solely or with a divisor (the cumulative GDD from sowing to sensing).

Once the yield prediction index or INSEY was established, different approaches for determining N requirements were proposed. For instance, a 5-step approach that required the prediction of the potential grain yield (using the INSEY index), grain N content, grain N uptake and early-season plant N uptake was proposed (Lukina et al., 2001); whereas a 7-step approach included, on top of the foregoing, the calculation of the predicted yield using the predicted potential grain yield, the response index and the forage N uptake (Raun et al., 2002). To refine the algorithm, consideration was equally given to variations in plant-stand densities (Raun et al., 2005; Teal et al., 2006). These approaches can be acted on and adapted in devising customized N fertilizer recommendations provided all inputs for building an algorithm are supplied.

The overall objective of this study was to improve barley N use efficiency through canopy sensing under Australia biophysical conditions by developing two algorithms for in-season N fertilizer prescriptions for barley grain and forage production. Upon validation, these algorithms may be used for N fertilizer recommendations on barley in Queensland (Australia). Possible outcomes of using the algorithm may include input cost reductions, limited environmental contamination, higher quality grains and forage and better financial returns.

Table 1. Soil analyses results.

Assay	Unit	Value
Sample depth	cm	0 - 15
Soil colour	-	Brown
Soil texture	-	Clay
pH (1:5 Water)	-	7.8
pH (1:5 $CaCl_2$)	-	6.8
Electrical conductivity (1:5 Water)	dS/m	0.09
Electrical conductivity (Saturated extract)	dS/m	0.6
Chloride	mg/kg	28
Organic carbon	%	0.8
Nitrate nitrogen	mg/kg	2
Ammonium nitrogen	mg/kg	2
Phosphorus (Colwell)	mg/kg	110
Phosphorus (BSES)	mg/kg	490
Phosphorus buffer index (PBI-Col)	-	76
Sulphate sulphur (MCP)	mg/kg	2
Cation exchange capacity	cmol(+)/kg	23.0
Calcium (Amm-acet)	cmol(+)/kg	12.0
Magnesium (Amm-acet)	cmol(+)/kg	9.9
Sodium (Amm-acet)	cmol(+)/kg	0.64
Potassium (Amm-acet)	cmol(+)/kg	0.65
Available potassium	mg/kg	250
Aluminium (KCl)	-	<0.1
Aluminium (KCl)	mg/kg	<9.0
Aluminium saturation	%	<1.0
Calcium % of cation	%	51.0
Magnesium % of cations	%	43
Sodium % of cations	%	2.80
Potassium % of cations	%	2.80
Calcium/Magnesium ratio	-	1.2
Zinc (DTPA)	mg/kg	0.85
Copper (DTPA)	mg/kg	1.00
Iron (DTPA)	mg/kg	28.0
Manganese (DTPA)	mg/kg	5.2
Boron (Hot $CaCl_2$)	mg/kg	0.5

MATERIALS AND METHODS

Experimental site and design

The experiment was conducted at the research facility of the University of Queensland located in Gatton Campus during the winter barley growing season, from June to October, 2016. The previous crop for the trial area was forage sorghum with no added fertilization in order to substantially deplete the soil N level. Soil test analyses were undertaken prior to sowing and the results are presented in Table 1. Based on these results, some nutrient deficiencies were corrected. Climatic conditions prevailing throughout the experiment were gathered by a weather station located within the premises of the research facilities. Downloaded from the Australian Bureau of Meteorology website (BOM, 2016), monthly averages of these climatic conditions are summarized in Table 2.

The area was ploughed and 16 subplots of 24 m^2 each were seeded at a sowing rate of 54 kg ha^{-1}, a sowing depth of 50 to 75 mm and an expected planting density of 60 plants m^{-2} (600,000 plants ha^{-1}). Corvette was the barley cultivar sown. This cultivar is grown in Queensland for both grain and forage production. Alleys between subplots were 2 m wide to minimize interferences between fertilizer treatments.

The trial was set up as a mono-factorial randomized complete block design with four treatments: N0, N50, N100 and N150, standing for 0, 50, 100 and 150 kg N ha^{-1}, respectively. With the exception of the control subplot (0 kg N ha^{-1}), N fertilizers were applied once prior to sowing (pre-plant fertilization). Weed control was performed by application of pre-emergence and post-emergence herbicide. Four weeks after emergence onwards, manual weeding was done weekly to reduce competition. Disease and pest control were unnecessary as attacks and infestations were insignificant. Irrigation was scheduled whenever water stress was likely to occur. An equivalent of 110 mm irrigation was supplied at 1, 2, 5 and 8 weeks after sowing (WAS).

Table 2. Climatic conditions prevailing during the experiment (Monthly means).

Months	Min. temp. (°C)	Max. temp. (°C)	Rainfall (mm)	9am relative humidity (%)	3pm Relative humidity (%)	9am Wind speed (km/h)	3pm Wind speed (km/h)
June	10.0	20.9	2.2	71.7	54.9	15.0	18.5
July	9.2	22.0	0.8	69.7	45.9	14.4	15.7
Aug.	7.1	22.6	1.1	69.6	44.8	10.9	15.2
Sept.	11.7	24.5	2.1	65.9	48.5	14.5	18.4
Oct.	9.2	27.9	1.0	46.1	34.5	16.2	15.7

Reflectance measurements

Canopy sensing was carried out across the whole subplot starting from 5 WAS at GS2 (Growth Stage 2 or Tillering) to 15 WAS at GS9 or ripening (Zadoks, 1974). The handheld GreenSeeker optical sensor unit (Trimble Navigation Limited) was used to measure the canopy reflectance. This sensor utilizes high intensity light emitting diodes (LED) and emits light in the red (660 ± 25 nm full width half magnitude, FWHM) and near infra-red (780 ± 25 nm FWHM) bands. A photodiode detector records the intensity of the reflected light. Electronic filters remove the soil background illumination and a multiplexed analogue-to-digital converter measures the filtered signal (Raun et al., 2002). Embedded software computes the reflectance in the red and near infra-red to output the NDVI. The sensor's field of view is an oval window of approximately 25 to 50 cm wide when held above the canopy at 60 to 120 cm, respectively. Sensing was operated by pulling the trigger at the start of rows and moving along them. Multiple readings are accumulated and an average was provided once the trigger was released at the end of the sensed area. The maximum measurement interval was 60 s. For optimal reading, the sensor was kept at a consistent heigh (60 cm) above the canopy and moved along rows at the speed of 2 m s^{-1} to keep the maximum measurement interval below 1 min. For each subplot, at least four NDVI average values were recorded and stored in an Excel spreadsheet.

Agronomic measurements and N analysis

Five different response variables (or first class variables) were measured: in-season forage N content (FNC_i), forage yield (FY), forage N content at harvest (FNC_h), grain yield (GY), and grain N content (GNC). These were then used to infer other values used to develop the algorithms.

Sampling for FNC_i and FNC_h was performed at 8 WAS (GS3 or stem elongation) and 15 WAS (GS9 or ripening), respectively. Samples were collected inside a square metre quadrat. The collection was done by handclipping the whole quadrat 2 cm above the ground. Samples were weighed, maintained in a drier at 65°C for 78 h, weighed again and then ground. The powder was thoroughly mixed and 250 mg sampled for N analyses. The N concentration was determined by the Dumas method (Bremner and Mulvaney, 1982) using the elemental analyzer Vario MACRO CHN/CHNS in the CAL Laboratory at UQ Gatton. The in-season forage N uptake and the forage N uptake at harvest were then calculated by multiplying the N concentration with the forage dry biomass.

Forage and grain harvest were performed at 15 and 16 WAS, respectively. An area of one square meter within each subplot was hand-clipped and the total biomass was collected, dried for 78 h at 65°C in a drier, then weighed to obtain forage dry biomass and consequently the FY. Another square meter within each subplot was hand-clipped and barley heads snipped, dried for 78 h at 65°C in a drier and then threshed to obtain barley grains. Grains were weighed to measure the GY and about 100 g of these were randomly sampled and ground. 250 mg of the thoroughly mixed powder were subsampled for GNC analyses using the Dumas method. Grain N uptake was calculated by multiplying the GNC by GY.

Statistical analysis and calculations

A number of second class variables were used to develop the algorithm: apparent N recovery (ANR), forage N uptake at harvest ($FNUP_h$), grain N uptake (GNUP), in-season forage N uptake ($FNUP_i$), in-season estimate of yield (INSEY), response index (RI) and normalized difference vegetation index (NDVI).

Apparent N recovery

$$\mathrm{ANR} = \frac{\mathrm{NUP_N} - \mathrm{NUP_0}}{\mathrm{N}} \times 100 \quad (1)$$

where ANR: apparent N recovery (%); NUP_N: N uptake at applied N rate (kg N ha^{-1}); NUP_0: N uptake at zero N rate (kg N ha^{-1}); N: applied N rate (kg N ha^{-1}).

Forage N uptake at harvest

$$\mathrm{FNUP}_h = \mathrm{FY} \times \mathrm{FNC}_h \quad (2)$$

where $FNUP_h$: forage N uptake at harvest (kg N ha^{-1}); FY: forage yield (kg ha^{-1}); FNC_h: forage N content at harvest (%).

Grain N uptake

$$\mathrm{GNUP} = \mathrm{GY} \times \mathrm{GNC} \quad (3)$$

where GNUP: grain N uptake (kg N ha^{-1}); GY: grain yield (kg ha^{-1}); GNC: grain N content (%).

In-season forage N uptake

$$\mathrm{FNUP}_i = \mathrm{DB}_i \times \mathrm{FNC}_i \quad (4)$$

where FNUPi: in-season forage N uptake (kg N ha^{-1}); DB_i: in-season dry biomass (kg ha^{-1}); FNC_i: in-season Forage N content (%).

Table 3. Barley response to four N rates for algorithms development.

N rates	FY (kg ha^{-1})	GY (kg ha^{-1})	FNUP$_i$ (kg N ha^{-1})	FNUP$_h$ (kg N ha^{-1})	GNUP (kg N ha^{-1})	RI	ANR (%)
0 kg N ha^{-1}	4,185^{c}	1,581^{c}	10.95^{b}	28.64^{b}	20.59^{c}	1.62^{a}	-
50 kg N ha^{-1}	9,215^{b}	4,086^{b}	27.11^{b}	65.34^{ab}	54.70^{b}	1.10^{b}	0.73^{a}
100 kg N ha^{-1}	1,1470^{a}	4,756^{a}	53.03^{a}	91.18^{a}	71.74^{a}	1.02^{c}	0.62^{a}
150 kg N ha^{-1}	1,1475^{a}	4,752^{a}	67.45^{a}	102.66^{a}	77.83^{a}	1.00^{c}	0.49^{ab}

Means within a column followed by different letters differ at p< 0.05 by the Least Significant Difference test (LSD). FY: Forage yield; GY: grain yield; FNUPi: in-season forage N uptake; FNUP$_h$: forage N uptake at harvest; GNUP: grain N uptake; RI: response index; ANR: apparent N recovery.

In-season estimate of yield

$$\mathrm{INSEY} = \frac{\mathrm{NDVI}}{\mathrm{NDS}} \quad (5)$$

Number of days from sowing to sensing where GDD is larger than zero. All over the growing season daily temperatures were larger than the GDD.

Response index

$$\mathrm{RI} = \frac{\mathrm{NDVI}_{rich}}{\mathrm{NDVI}_{sowing}} \quad (6)$$

where RI: response index; NDVI$_{rich}$: NDVI measured in a N rich strip, and N is non-limiting; NDVI$_{sowing}$: NDVI measured in the plot with the sowing N rate. The sowing N rate can range from zero to the level where the N is non-limiting.

Normalized difference vegetation index

$$\mathrm{NDVI} = \frac{\mathrm{R-NIR}}{\mathrm{R+NIR}} \quad (7)$$

where NDVI: normalized difference vegetation index; R: canopy reflectance in the red band (660±25 nm); NIR: canopy reflectance in the near infra-red band (780±25 nm).

Parametric statistical analyses were performed using the R version 3.3.1 released 21-06-2016. Three major steps were followed: (1) check the normality of response variables, (2) test the significance of differences among treatment means using the least significant difference (LSD) test, and (3) test the significance of correlation coeficients, regression coeficients and the regression models. All variables were submited for analysis of variance (ANOVA) to assess the statistical significance of treatment effects across the four treatments and then means were compared for significant differences. Simple linear regression analyses based on ordinary least square (OLS) estimation were performed to describe the relationship between the independent variables (NDVI readings) and dependent variables (yield and N contents). The level of significance throughout these statistical analyses was set at α = 0.05.

RESULTS AND DISCUSSION

Barley response to N rates

All agronomic variables measured responded positively to different N rates (Table 3). With the exception of the two highest N rates where no significant differences were noticed, FY, GY, FNUP$_i$, FNUP$_h$ and GNUP significantly increased with increasing N rates, thus delivering enough variability in data to make possible the derivation of N fertilizer algorithms.

Canopy reflectance and N rates

Barley canopy reflectance increased with increasing N levels. With the exception of 5 and 6 WAS where no significant differences were detected between treatments, higher N rates induced higher NDVIs (Figure 1). N fertilization fosters biomass production and enhances leaf greenness. Being an index specific for chlorophyll and vegetation discrimination, NDVI is sensitive to both green and dense canopies (Peñuelas et al., 1997). In canopy reflectance of barley at various N rates under two irrigation regimes, Kleman and Fagerlund (1987) equally noticed that reflectance increased with increasing N rates as a result of biomass and leaf pigment accumulation. Furthermore, when NDVI was regressed against N rates, strong coefficients of determination were observed at all growth stages (Figure 2) except for weeks 5 to 7, therefore reinforcing the assumption that barley NDVIs can be used for indirect assessment of pre-plant N levels between 7 and 15 WAS.

The inability of NDVI in capturing N levels at earlier growth stages (5 to 7 WAS) may be attributable to the open canopy. In effect, at incomplete canopy cover, NDVI is affected by soil background optical properties (Bausch, 1993). Similar conclusions were reached upon comparing the effects of different soil backgrounds at various vegetation cover and this influence was more pronounced on lighter coloured soils than on dark agricultural soils (Elvidge and Lyon, 1985; Huete et al., 1985). Thus, in the current study it appears that lower population densities interfered with NDVI reliability, limiting its early season biomass and N status estimations. Alternatives such as delaying reflectance measurements until near canopy closure or augmenting population density could be explored to improve NDVI reliability at these early stages of crop development.

Barley NDVI seemed to approach its saturation point between 100 and 150 kg N ha^{-1}, endorsing this commonly

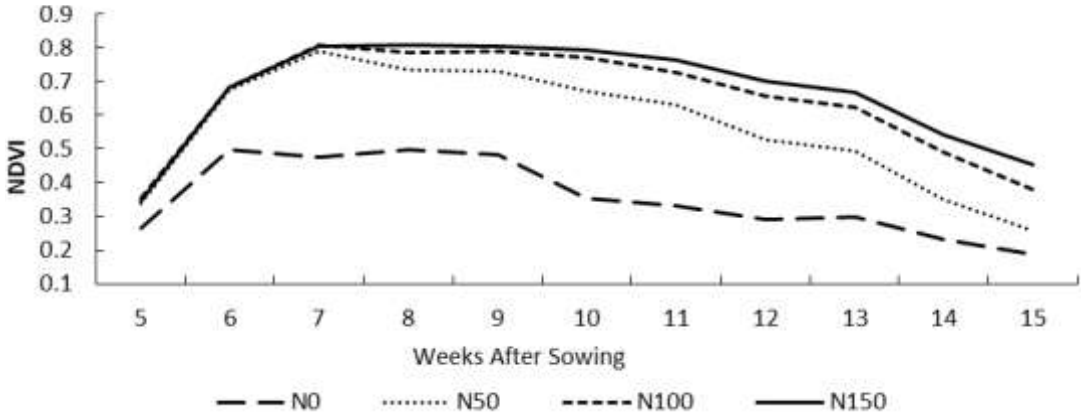

Figure 1. Barley NDVI fluctuation across the growing season at four N rates. N0: 0 kg N ha^{-1}, N50: 50 kg N ha^{-1}, N100: 100 kg N ha^{-1}, N150: 150 kg N ha^{-1}.

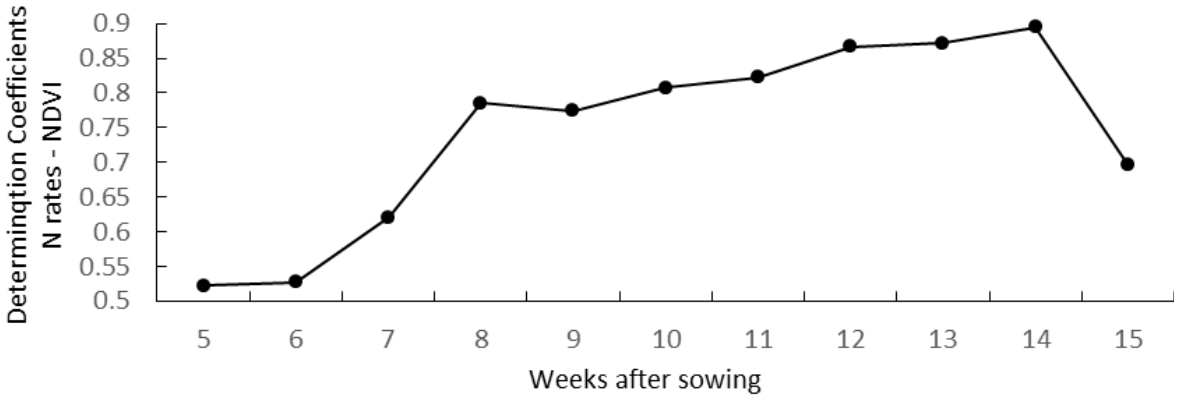

Figure 2. Fluctuation of determination coefficients between N rates and barley NDVI over the growing season.

known NDVI weakness of reaching saturation prematurely as compared to other vegetation indices (Figure 1). NDVI saturation usually occurs when at larger leaf area indices rather than increasing linearly, NDVI readings asymptotically reach a constant value (Haboudane et al., 2004). Because NDVI saturation usually occurs under dense canopies (Gu et al., 2013), this suggests that the population density and the two highest N treatments tested in the current experiment induced NDVI near-saturation. Thus, at N rates exceeding 150 kg N ha^{-1} and sowing densities above 600,000 plants per hectare, NDVI is expected to reach saturation thereby rendering it a poor estimator of barley biomass and N content.

Over the growing season, NDVI exhibited a parabolic-shape curve irrespective of N rates. For instance, at 150 kg N ha^{-1}, NDVI steadily increased from 0.35 at one WAS to peak of 0.81 at eight WAS, then decreased to a lower value (0.45) at the ripening stage (GS9). Apart from the declining phase of the NDVI curve for the past week 9, this pattern resembles the N uptake curve and the biomass production curve of barley over time (Lemaire et al., 2008; Whitmore, 1988). Indeed, under steady N supplies, most cereal crops expand their canopies, accumulate biomass and store N in leaves in the form of Rubisco or Ribulose-1,5-bisphosphate carboxylase/oxygenase, a compound produced during photosynthesis (Millard, 1988). Once maximum leaf area and biomass are attained, the N uptake is restricted to covering deficits resulting from grain filling and limited remobilization from senesced leaves (Jeuffroy and Bouchard, 1999). Thus at anthesis and post-anthesis stages (GS5 or week 11 and beyond), leaf biomass, leaf N content and forage N uptake decline until complete senescence of the canopy occurs. Being an index sensitive to chlorophyll content, biomass and leaf area, NDVI mirrored these physiological processes over the growing season.

Canopy reflectance, yield and N uptake

Barley NDVI correlated well with both N uptake and yield. When NDVI was regressed against $FNUP_i$ at eight WAS,

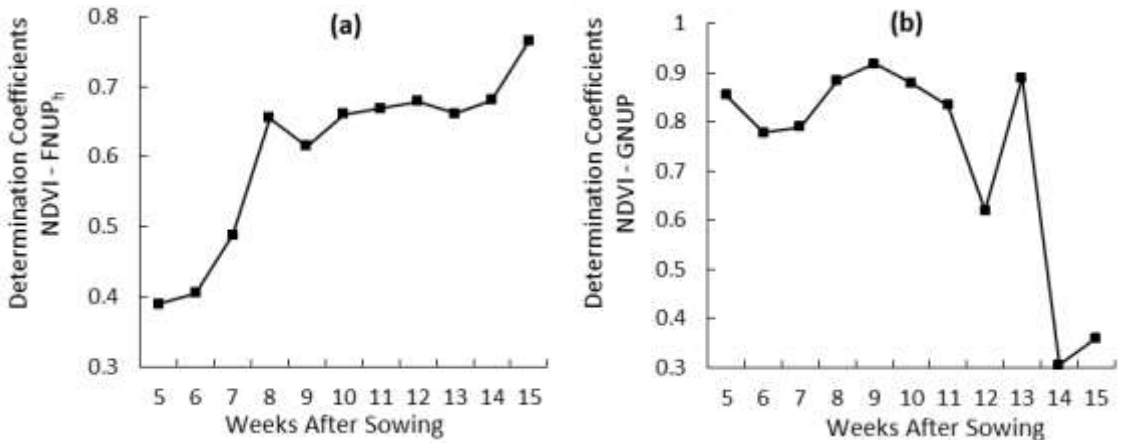

Figure 3. Fluctuation over the growing season of determination coefficients between NDVI and Forage N uptake at harvest ($FNUP_h$) (a) and NDVI and grain N uptake (GNUP) (b).

a fairly strong determination coefficient was noticed (R^2 = 0.63, $p < 0.001$) confirming the assumption of a linear relationship. The correlation was equally positive for both components of N uptake, namely dry biomass (R^2 = 0.58, $p<0.001$) and N concentration (R^2 = 0.74, $p<0.001$). Earlier studies on wheat ascertained that NDVI not only correlated with total N uptake but equally with N content (Sembiring et al., 2000; Wright et al., 2004). Just as NDVI is used as estimator of in-season N uptake on wheat the same is achievable on barley.

It was equally noted that NDVI at GS3 displayed a positive linear correlation with $FNUP_h$ (R^2 = 0.65, $p<0.001$) and GNUP (R^2 = 0.84, $p<0.001$). Assessments over the growing season of determination coefficient fluctuation of NDVI with $FNUP_h$ and GNUP revealed that from 7 to 15 WAS NDVI could provide an accurate estimation of $FNUP_h$ and GNUP as these coefficients were at least equal to 0.50 (Figure 3). Additionally, upon correlating NDVI with FY and GY, determination coefficients were almost always above 0.50 (Figure 4). It seems that the fairly high correlations of NDVI with end season N uptakes in grain, forage and yields at nearly all sensed growth stages could be due to favorable climatic and biotic conditions. Nevertheless, in a practical sense, only stages in which split N application is possible will be of interest for algorithm development. Therefore, barley NDVI at 8 WAS becomes reliable in predicting barley $FNUP_i$, FY, $FNUP_h$, GY and GNUP, and as such, can be used in developing an in-season fertilizer algorithm, especially because side dressing can be performed at that stage.

Development of sensor-based algorithms for in-season N fertilization on barley

Various approaches have been adopted in developing sensor-based algorithms for in-season N side dressing. Only one of them is discussed for barley forage and grain production in Queensland (Australia). Developed on winter wheat (Raun et al., 2005), this approach required the following inputs: prediction equations for the potential yield based on the NDVI in-season estimate of yield (INSEY); the prediction of N content in grain or forage; the N uptake estimation in grain or forage; and the in-season crop N uptake. Beside these four equations, two additional parameters included the response index (RI) which is the magnitude of the barley response to N fertilizer in-season and the apparent N recovery (ANR). To determine how much N needs to be applied in-season to barley, the knowledge of the potential yield (PY) is a prerequisite. Indeed, knowing the potential yield enables the inference of total N required to achieve that target yield. The potential yield can be estimated early in the growing season through canopy reflectance. Since NDVI exhibited a high determination coefficient with FY (R^2 = 0.84, $p<0.001$) and GY (R^2= 0.88, $p<0.001$), especially from 8 WAS, this relationship can be used for its prediction. Based on in-season barley canopy reflectance at 8 WAS (GS3), it was possible to obtain an equation relating grain and forage yield as a function of INSEY (Figure 5). INSEY was computed by dividing the NDVI value at GS3 by the number of days from sowing to sensing. Predictive equations for forage and grain PY are as follows:

$$PY_{forage} = 0.7841 \times e^{278.81 \times INSEY} \quad (8)$$

where PY_{forage}: predicted forage potential yield (Mg dry biomass ha^{-1}); INSEY: in-season estimate of forage yield.

$$PY_{grain} = 0.2558 \times e^{306.53 \times INSEY} \quad (9)$$

where PY_{grain}: predicted grain potential yield (Mg dry biomass ha^{-1}); INSEY: in-season estimate of grain yield.

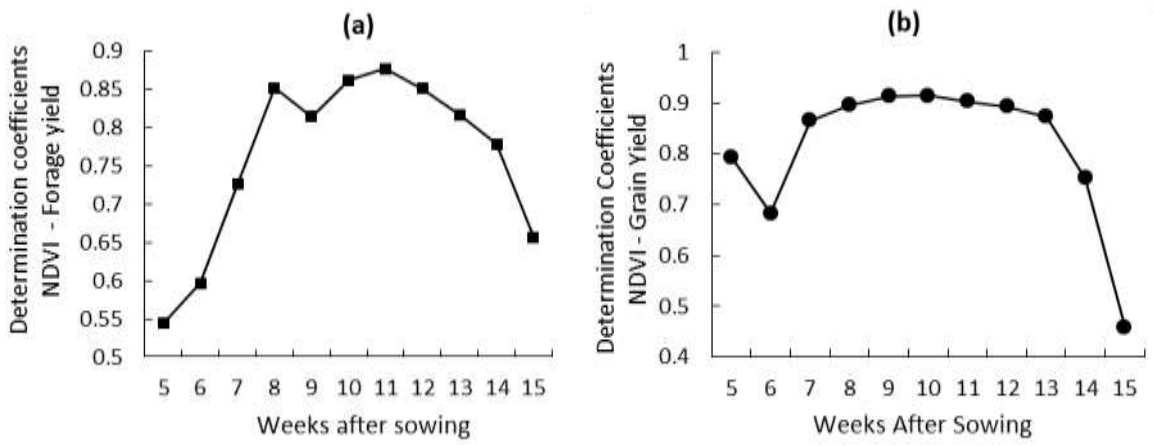

Figure 4. The fluctuation over the growing season of determination coefficients between NDVI and forage yield (a) and NDVI and grain yield (b).

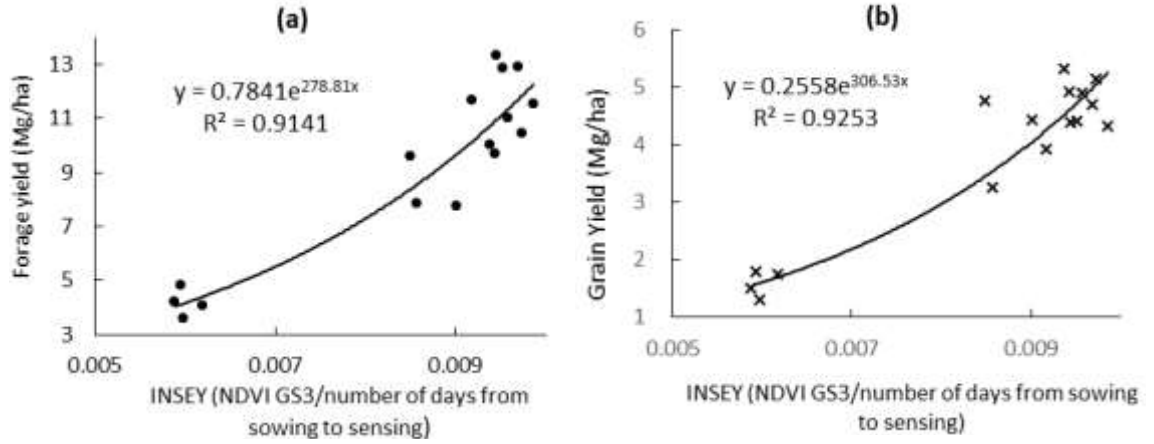

Figure 5. Relationship between in-season estimate of yield (INSEY) and forage yield (a) and in-season estimate of yield and grain yield (b).

The knowledge of the potential yield is not enough to determine how much N should be applied because N uptake may vary greatly from season to season depending on soil N dynamics. Unfortunately, predicting soil N availability is complex. Still, the crop itself can provide an indirect assessment of soil N through its response to N fertilization. Various studies have established that under restricted conditions, N uptake in cereal crops is higher when N is supplied than when under sufficiency conditions (Lemaire et al., 2008). Thus predicting the crop response to N supply fosters N efficiency. The response index (RI) was proposed to take into account crop response to N fertilization (Mullen et al., 2003). The crop RI is inferred through sensing and computed by dividing the NDVI of a non-limiting N strip with the NDVI of the field with the pre-sowing N rate. In this experiment, 150 kg N ha^{-1} appeared to be the non-limiting N rate. At that rate, lodging was noticed, an indication of excess N supply. The adjusted predicted potential yield (APY) equals PY times RI, and is expressed as follows:

$$APY_{forage} = RI \times 0.7841 \times e^{278.81 \times INSEY} \quad (10)$$

where APY_{forage}: adjusted predicted forage potential yield based on RI (Mg dry biomass ha^{-1}); RI: response index; INSEY: in-season estimate of forage yield.

$$APY_{grain} = RI \times 0.2558 \times e^{306.53 \times INSEY} \quad (11)$$

where APY_{grain}: adjusted predicted grain potential yield based on RI (Mg dry grain.ha^{-1}); RI: response index; INSEY: in-season estimate of grain yield.

The next step in the algorithm development consisted of predicting N content in forage and grains based on the predicted APY. A polynomial relationship was

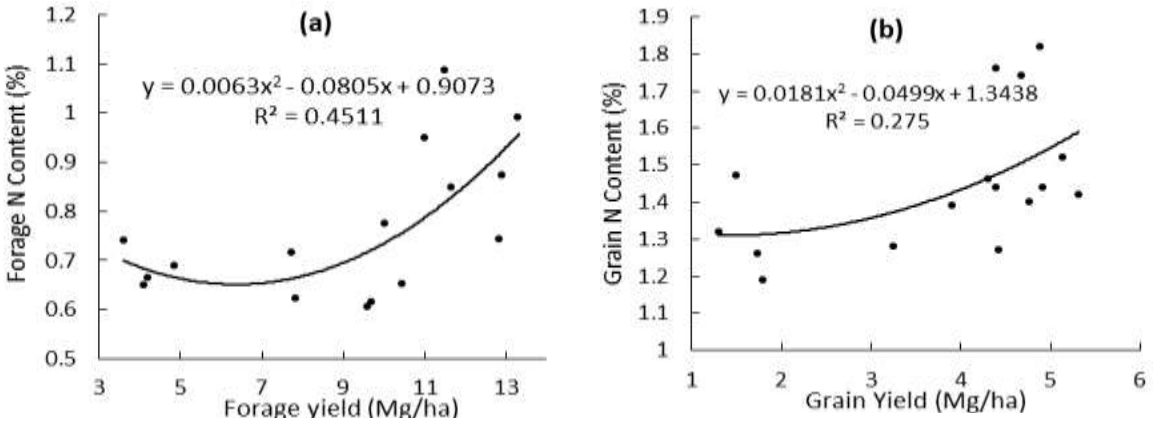

Figure 6. Relationship between forage yield and forage N content (a) and grain yield and grain N content (b).

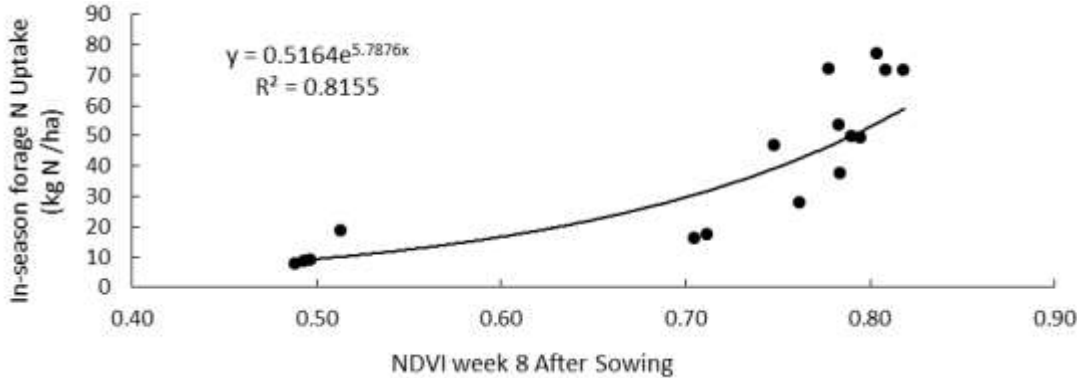

Figure 7. Relationship between NDVI and in-season forage N uptake .

established between yield and N content (Figure 6) and used to predict N content on harvested parts as:

$$PNC_{forage} = 0.0063 \times (APY_{forage})^2 - 0.0805 \times (APY_{forage}) + 0.9073 \quad (12)$$

where PNC_{forage}: predicted N content in forage (%); APY_{forage}: adjusted predicted forage potential yield based on RI (Mg dry grain.ha^{-1}).

$$PNC_{grain} = 0.0181 \times (APY_{grain})^2 - 0.0499 \times (APY_{grain}) + 1.3438 \quad (13)$$

where PNC_{grain}: predicted N content in grain (%); APY_{grain}: adjusted predicted grain potential yield based on RI (Mg dry grain ha^{-1}).

The prediction of N content helps in computing the predicted grain or forage N uptake, which is the product of the PNC and the APY.

$$FNUP = PNC_{forage} \times APY_{forage} \times 10^{-2} \quad (14)$$

where FNUP: forage N Uptake (Mg N ha^{-1}); PNC_{forage}: predicted N content in forage (%); APY_{forage}: adjusted predicted forage potential yield based on RI (Mg dry biomass ha^{-1}).

$$GNUP = PNC_{grain} \times APY_{grain} \times 10^{-2} \quad (15)$$

where GNUP: grain N Uptake (Mg N ha^{-1}); PNC_{grain}: predicted N content in grain (%); APY_{grain}: adjusted predicted grain potential yield based on RI (Mg dry grain ha^{-1}).

The second last step in the algorithm development requires predicting the early or in-season crop N uptake(also $FNUP_i$). The $FNUP_i$ corresponds to the amount of N extracted in the soil by barley from sowing to sensing. This quantity has to be subtracted from $FNUP_h$ or GNUP which indicate the total N taken up from sowing to harvest. The prediction equation of $FNUP_i$ as a function of NDVI was determined (Figure 7) and it reads:

$$FNUP_i = 0.516 \times e^{5.78 \times NDVI} \quad (16)$$

where $FNUP_i$: in-season forage N uptake (kg N ha^{-1}) at GS3; NDVI: normalized difference vegetation index

measured at GS3.

Finally, the N fertilizer requirement (FNR) is deduced by subtracting $FNUP_i$ from FNUP or GNUP and dividing the difference by the apparent N recovery (ANR) to account for the fact that not all N applied is taken up by the barley crop. During this study, the ANR averaged 62%. Thus, the in-season N requirement based on sensing barley canopy at GS3 (8 WAS) is obtained by these equations:

$$\mathrm{FNR}_{\mathrm{forage}} = \frac{\mathrm{FNUP}_h - \mathrm{FNUP}_i \times 10^{-3}}{0.62} \quad (17)$$

FNR_{forage}: fertilizer N requirements for forage production (Mg N ha^{-1}); $FNUP_h$: forage N uptake at harvest (Mg N ha^{-1}); $FNUP_i$: In-season forage N uptake (kg N ha^{-1}).

$$\mathrm{FNR}_{\mathrm{grain}} = \frac{GNUP - \mathrm{FNUP}_i \times 10^{-3}}{0.62} \quad (18)$$

where FNR_{grain}: fertilizer N requirements for grain production (Mg N ha^{-1}); GNUP: grain N uptake (Mg N ha^{-1}); $FNUP_i$: in-season forage N uptake (kg N ha^{-1}).

Conclusions

The low efficiency of N fertilizer threatens farmers' financial returns but most importantly the environment. Among the existing strategies being developed to address the matter, crop canopy sensing holds an advantageous position, and is being considered a promising tool in precision agriculture. The current study developed two distinct algorithms: one for barley forage production and another for grain production in Queensland using the handheld GreenSeeker sensor. The sensor measured barley canopy reflectance over the growing season and output NDVI values. These values were correlated with yields (grain and forage) and with N uptake (in-season and at harvest). The power of these correlations was high enough to justify the design of in-season N fertilizer algorithms. The developed algorithms were expected to safeguard natural ecosystems through minimization of unused N but also to improve financial returns of barley growers through reductions of N related costs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 13(29), pp. 1487-1495, 19 July, 2018
DOI: 10.5897/AJAR2018.13241
Article Number: D863D6C57850
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Screening of cowpea (*Vigna unguiculata* (L.) Walp.) lines for resistance to three Aphids (*Aphis craccivora* Koch) strains in Burkina Faso

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Received 14 May, 2018; Accepted 18 June, 2018

Cowpea (*Vigna unguiculata* L. Walp.) is an important cash, food and nutritional security grain legume crop in the semi-arid regions of sub-Saharan Africa. However, its productivity is hampered by several biotic stress factors including numerous insect pests that infest and damage the crop at all its development stages in the field as well as during storage. This study sought to identify new sources of resistance to cowpea aphids. Ten lines were infested with three strains of aphids and their resistance was evaluated. Results revealed that among the cowpea genotypes, line IT97K-556-6 was found to be the most resistant to all the three strains of aphids. Line N ° 2300 was the most susceptible to all the three strains of aphids. KN-1 considered as a susceptible control was found to be resistant. Prior to lines classification based on their resistance to aphids, the analysis of the Area Under the Infestation Progress Curve (AUIPC) showed that IT97K-556-6 has exhibited the higher level of antibiosis. It was the least favorable to the development of aphids and NS-1 was the most favorable. Promising lines have been identified for further evaluation and utilization for improvement in cowpea.

Key words: *Vigna unguiculata, Aphis craccivora,* antibiosis, genetic resistance, area under the infestation progress curve (AUIPC), control.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L) .Walp. is a proteinaceous plant with a high nutritional value. Like other legumes, awareness of its nutritional benefits, its importance in food security and sustainable agriculture, and in mitigating biodiversity loss and climate change have been promoted by the United Nations General Assembly who designated 2016 the International Year of Pulses (LPWG, 2017). It is the most economically important indigenous African

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Table 1. Aphids collecte zones climatic features.

Climatic zone (Site)	Sudanian (Farakoba)	Sudano-Sahelian (Sà)	Sahelian (Sambonaye)
Annual average rainfall (mm)	1015,8	755,3	442,6
Annual average potential evapotranspiration (mm)	2011,1	2444,0	2027,0
Annual average temperature (°C)	27,3	28,7	29,7

Source : Pallo and Sawadogo (2010).

legume crop (Langyntuo et al., 2003). Cowpeas are of vital importance to the livelihood of several millions of people in West and Central Africa. Rural families that make up the larger part of the population of these regions derive from its production, food, animal feed, alongside cash income (Gómez, 2004). West Africa alone produces about 83% of the world production (Faostat, 2016). However, grain yield remains the lowest in these areas. It rarely exceeds 400 to 500 kg per hectare in the traditional production system (Langyintuo et al., 2003). High yields in cowpea are seriously militated by insect pests and without insect control, yields range between 100 and 250 kg ha-1(Oladejo et al., 2017).

The cowpea aphid, *Aphis craccivora* Koch (Homoptera, Aphididae) is one of the insects causing serious yield losses in cowpea. This species feeds on phloem and became a major pest of cowpea in Africa, Asia and America (Obeng-Ofori, 2007). Aphids cause damage to susceptible cultivars directly by modifying the metabolism and by extracting plant nutrients and, indirectly, by transmitting phytopathogenic viruses (Blackman and Eastop, 2000). At least 14 legume viruses are transmitted by *A. craccivora* (Thottapilly et al., 1990). Host plant resistance is an efficient and environmentally friendly way of controlling insects, including aphids. Some cowpea lines have been screened in Africa and different levels of resistance to aphids have been found (Kusi et al., 2010; Souleymane et al., 2013; Aliyu and Ishiyaku, 2013, Omoigui et al., 2017). Unfortunately resistance-breaking biotypes have occurred in several plant–aphid systems (Dogimont et al., 2010). So, new resistant sources need to be developed and deployed in cowpea growing areas where aphids occur in order to reduce the risk of resistance break-down. Genetic studies on aphid resistance have also been done (Bata et al., 1987; Pathak and Krishna, 1991; Huynh et al., 2015) but the results of these studies differ from one to another raised questions about the mode of inheritance to aphids. Thus, to increase the understanding of aphid resistance, it is necessary to find out more sources of resistance.

The purpose of this study was to contribute to the increase in the yield of cowpea by the identification of aphid resistant lines. The specific objectives were i) to assess differences among lines in terms of degree of aphids infestation ii) to find out relevant parameters to be used for lines classification and iii) to classify the lines based on their level of resistance to aphids.

MATERIALS AND METHODS

The screening test conducted in February 2014 was hosted by the laboratory of genetics and biotechnologies and the laboratory of entomology located at the Environmental, Agricultural Research and Training Center of Kamboinsé in Burkina Faso. The site geographical coordinates are: 12° 45’ North -1° 55’ West. Ten cowpea lines (B301; KVx295-2-124-99 SARC1-91-1 SARC1-57-2 IT97K-556-6 NS1 N°2300 NS-Farako-Ba CB27 and KN-1) were used for screening; among which IT97K-556-6 (resistant) and KN-1 (susceptible) lines were used as controls.

Three strains of aphids from Bobo-Dioulasso, Kamboinse and Pobé-Mengao were used for screening. Bobo-Dioulasso belongs to the Sudanian zone with temperatures ranged between 18.9 and 37.0°C. Kamboinse Belongs to the Sudano-Sahelian with mide-range temperatures (from 16.0 to 39.3°C) and Pobé-Mengao to the Sahelian zone with temperatures ranged between 14.3 and 42.4°C (Direction Générale de la Météorologie du Burkina Faso, 2016) (Figure 1).

The Sahelian zone is located in the North. It is the zone with the lowest rainfall in the country. The Sudano-Sahelian zone comprises of the most extensive climatic zone as it extends over all of the central part of the country. The Sudanian zone occupies the southern part of the country, where the rainy season lasts from 5 to 6 months. More details features of these climatic zones are displayed in Table 1.

The collection of aphids took place in October 2013. The stocks of insects brought from the fields were kept in rearing cages on susceptible line KVX 396-4-5-2D. The screening test for resistance was done with the offspring of the wild-type strain. Each strain was kept in a separate cage from the others to avoid mixing the 3 strains.

The experimental design was a Randomized Complete Block Design with three (3) blocks, five (5) replications each. Plants in each block were infested with only one of the three (3) aphid strains. The screening was done inside transparent plastic buckets. Seven days seedlings were used for this screening. The infestation consisted of taking a sample of five aphids and depositing them with a brush on each plant. Plants were regularly watered by direct water supply to the foot and remained under parasitic pressure throughout the duration of the test. For this, the upper part of each bucket was covered with insect (aphids)-proof tissue held by small wooden beams. The set was hermetically tied up using elastic ribbons. The bucket bases have been provided with small holes to facilitate the evacuation of irrigation water.

Data collection

Observations were made every three days and consisted of measuring some parameters: i) The number of trifoliate leaves; ii) The number of plants surviving; iii) The dynamics of the aphid populations using six scores ranged from 0 to 5(0 = No aphid, 1=1 to 5 aphids, 2 = 5 to 20 aphids, 3 = 20 to 100 aphids, 4 = 100 to 500 aphids, 5 = more than 500 aphids). This method of collection was inspired by that of Souleyman et al. (2013).

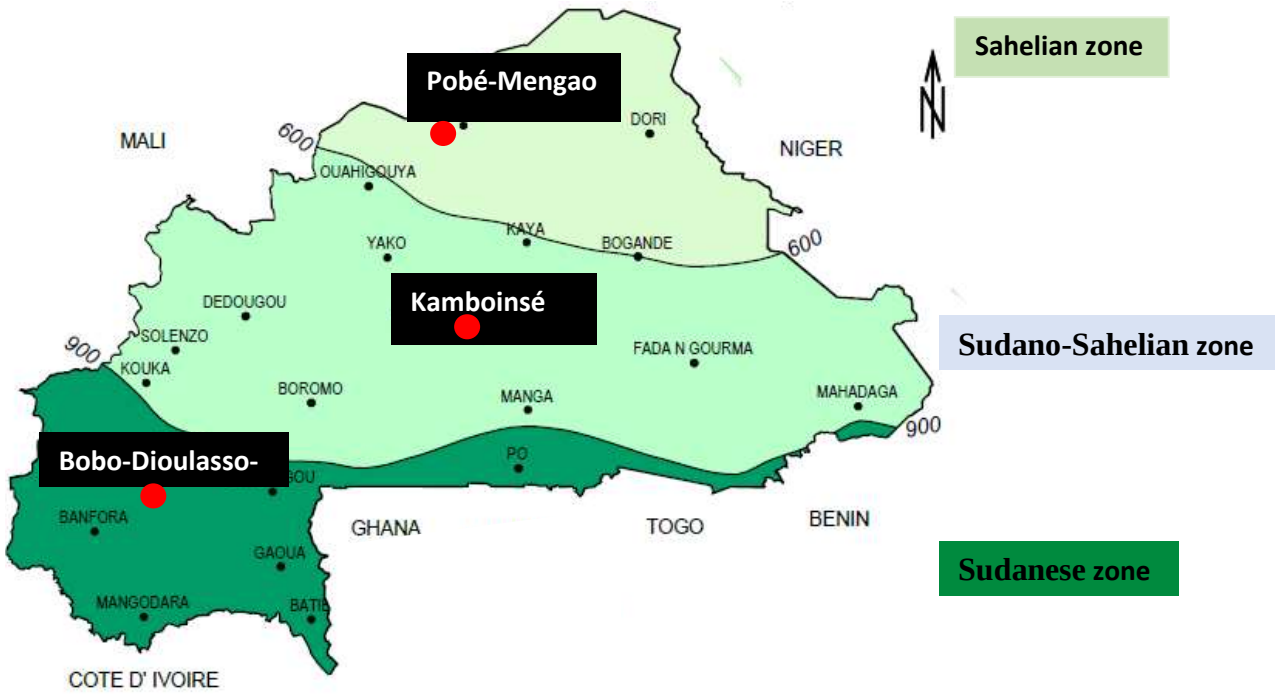

Figure 1. Climatic zones of Burkina Faso (1981 - 2010).
Source: Direction Générale de la Météorologie du Burkina Faso.

Data analysis

The raw data of one month after infestation and data of the (Area under the Infestation Progress Curve (AUIPC) were used for analyses. Analysis of variance (ANOVA) for the area under the infestation progress curve (AUIPC) was conducted to check first, whether the difference among lines based on their ability to slow down the multiplication of aphids is significant then, whether the difference among aphids strains is significant and finally to check the existence of line-strain interaction. SAS 9.4 software was used to analyze the data. The formula used to compute the AUIPC is:

$$AUIPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i) / 2] [T_{i+1} - T_i]$$

(Shaner et Finney, 1977)

X_i = Degree of infestation at the i^{th} observation
X_{i+1} = Degree of infestation at the $(i+1)^{th}$ observation
T_i = Time in days for the i^{th} observation
T_{i+1} = Time in days for the $(i+1)^{th}$ observation
n= Total number of observations

The raw data of one month after infestation was summarized to get the variables (Degree of infestation, Survival rate and the number of trifoliate leaves) means for each line. Using the same raw data, correlation analysis through the principal component analysis was conducted prior to the lines classification to investigate the relationship among variables in order to select the relevant variables. These selected variables were analyzed to determine the resistant lines. XLSTAT 7.1 was used to compute the data.

Hierarchical clustering analysis with three classes was conducted to identify which line is resistant, moderately resistant or susceptible using the raw data of one month after infestation with only the most important variables selected before. XLSTAT 7.1 was also used for this analysis.

RESULTS

Summary of variables mean values

Prior to more precise classification, Figure 2 is displaying the average performance of the lines under aphid's infestation. It represents the mean values of number of leaves, degree of infestation and survival rate one month after infestation. Line IT97K-556-6 has shown the highest survival rate (93%), the lowest degree of infestation (1.4) and an average number of leaves (11). Inversely, line N°2300 has shown the lowest survival rate (33%), a degree of infestation (3.6) and a low number of leaves (4). The figure is also showing that line KN-1 has recorded the highest number of leaves (20), a good survival rate (87%) and an average degree of infestation (2).

Similarity tests

In Table 2 the results of the analysis of variance (ANOVA) for the area under the infestation progress curve (AUIPC) are presented. These results are showing that the differences among lines ($P = 0.001$) and aphid

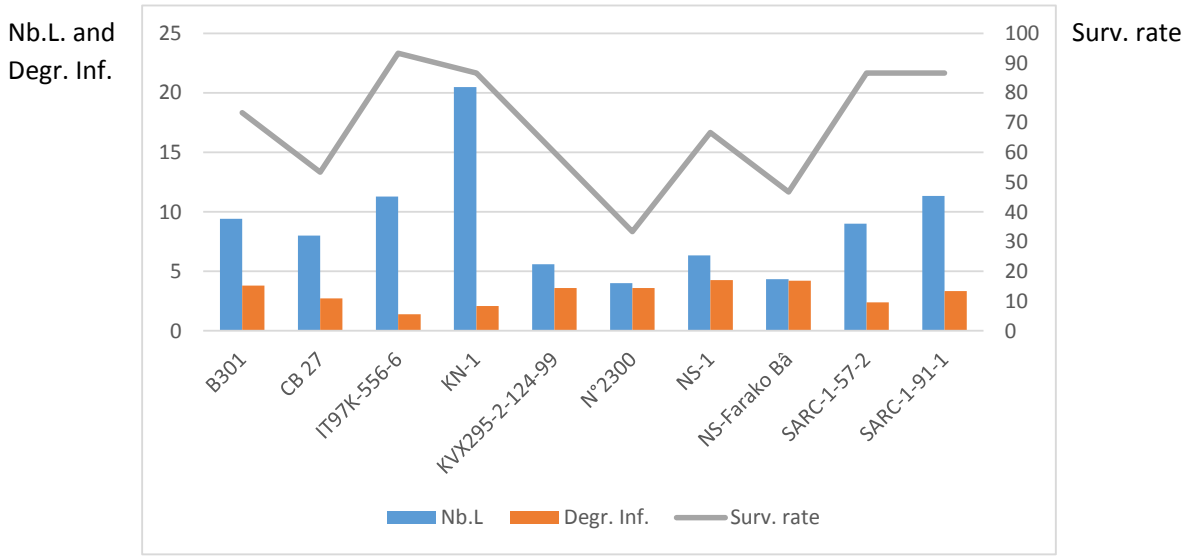

Figure 2. Mean values of number of leaves, degree of infestation and survival rate. Nb.L: number of leaves; Degr. inf: Degree of infestation; Surv. rate: survival rate.

Table 2. Analysis of variance (ANOVA) for the area under the infestation progress curve (AUIPC).

Fixed term	Wald Statistic	n.d.f.	F statistic	d.d.f.	F pr
Strain	10.22	2	5.11	120	**0.007****
Lines	43.83	9	4.87	120	**0.001****
Strain*Lines	18.27	18	1.01	120	0.448

Table 3. Correlations among the 3 variables (number of leaves, survival rate and degree of infestation).

Variables	*Nb.L*	*Degr.inf.*	*Surv. rate.*
Nb.L	1		
Degr.inf.	**-0,652**	1	
Surv.rate	**0,728**	**-0,628**	1

Nb.L: number of leaves; Degr. inf: Degrees of infestation; Surv. rate: survival rate.

strains (P = 0.007) were highly significant, while the interaction strain-line was not significant (P = 0.448).

Correlation test

These mean values were used to establish correlations among variables displayed in Table 3. These results are showing that the variable “number of leaves” was positively correlated with the “survival rate” and negatively correlated with the “degree of infestation” while the “survival rate” was negatively correlated with the “degree of infestation”.

Lines classification

Using the Hierarchical clustering three categories of lines (resistant, moderately resistant and susceptible) have been found (Figures 3, 4 and 5). Screened with the aphids’ strain of Kamboinsé the lines were regrouped as follows: N°2300, NS-1 and NS-Farakoba were susceptible, BC27 and SARC-1-57-2 were moderately resistant and IT97K-556-6, KN-1, KVX295-2-124-99,

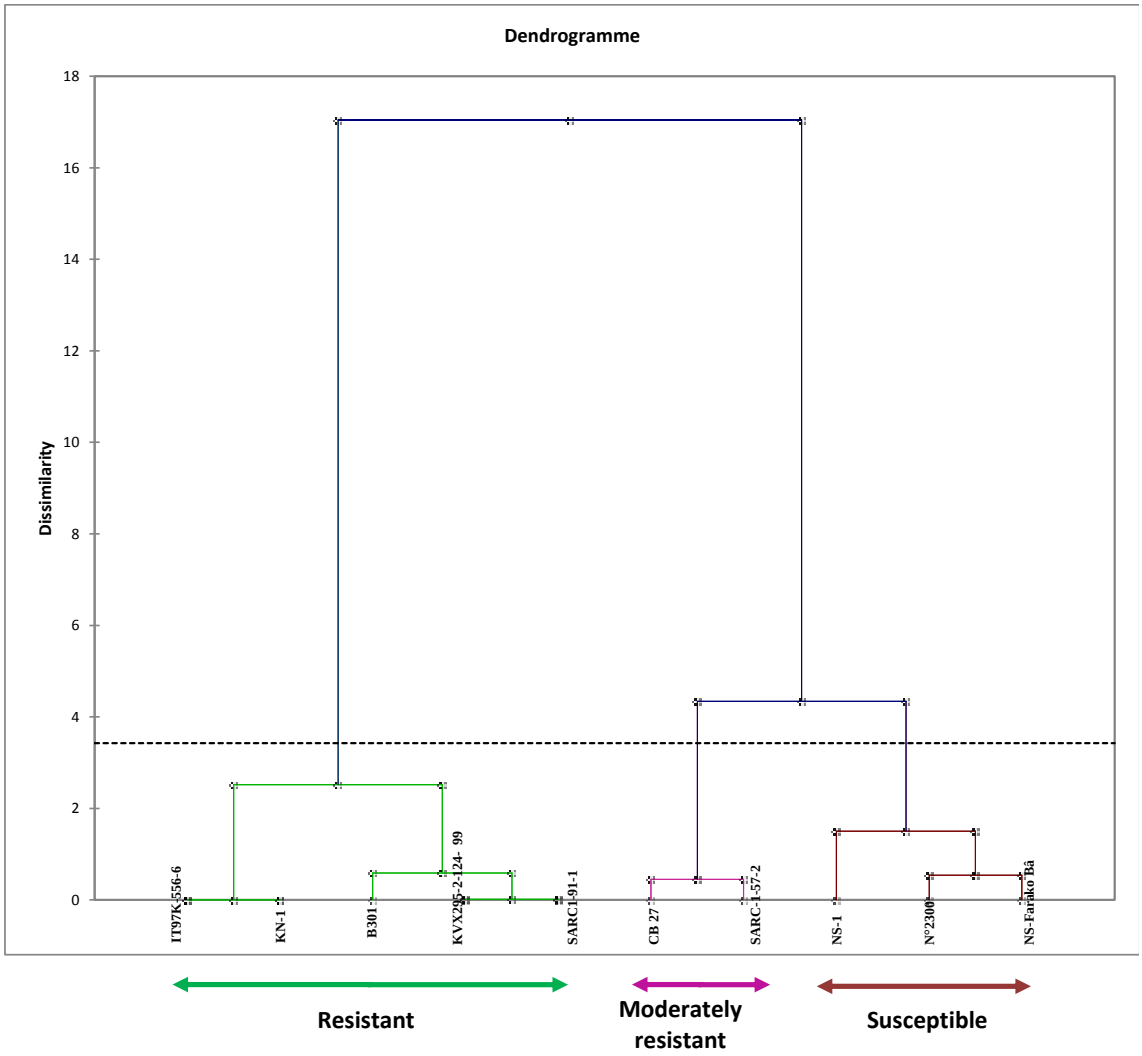

Figure 3. Dendrogram representing the three classes of lines (strain of kamboinse).

SARC1-91-1 were resistant (Figure 3). Screened with the aphids' strain of Bobo-Dioulasso, CB27, IT97K-556-6 and SARC1-91-1 were resistant. B301, KN-1, NS-1, NS-Farako Bâ and SARC-1-57-2 were moderately resistant and KVX295-2-124-99 and N°2300 were susceptible (Figure 4). As far as the strain of Pobé-Mengao is concerned IT97K-556-6, KN-1, KVX295-2-124-99 and SARC-1-57-2 were resistant, B301 and SARC1-91-1 were moderately resistant and CB 27, N°2300, NS-1 and NS-Farako Bâ were susceptible (Figure 5). Table 4 is a summary of Figures 2, 3 and 4. It is showing the lines status based on their level of resistance to aphids.

DISCUSSION

This preliminary analysis showed that line IT97K-556-6 is the highest resistant lines since it recorded the highest survival rate, the lowest degree of infestation and an average number of leaves. Line N°2300 by having the lowest survival rate (33%), a degree of infestation (3.6) and a low number of leaves (4) was considered to be the most susceptible line. It can also be noted that line KN1 has shown a better performance than expected.

The analysis of the AUIPC shows that the difference among lines is highly significant (P = 0.001). The capacity of multiplication of aphids depends upon the genotype of the line on which they develop. These results are in accordance with those of Laamari et al. (2008); Obopile and Ositile (2010) and Aliyu and Ishiyaku (2013). The difference among lines with regard to the area under the infestation progress curve is due to the capacity of some lines to slow down the development and multiplication of aphids by "antibiosis" (Teetes, 2007; Alabi et al., 2012; Omoigui et al., 2017). Thus, IT97K-556-6 has exhibited the highest level of antibiosis. It was the least favorable to the development of aphids and NS-1 was the most favorable. According to Teetes (2007),

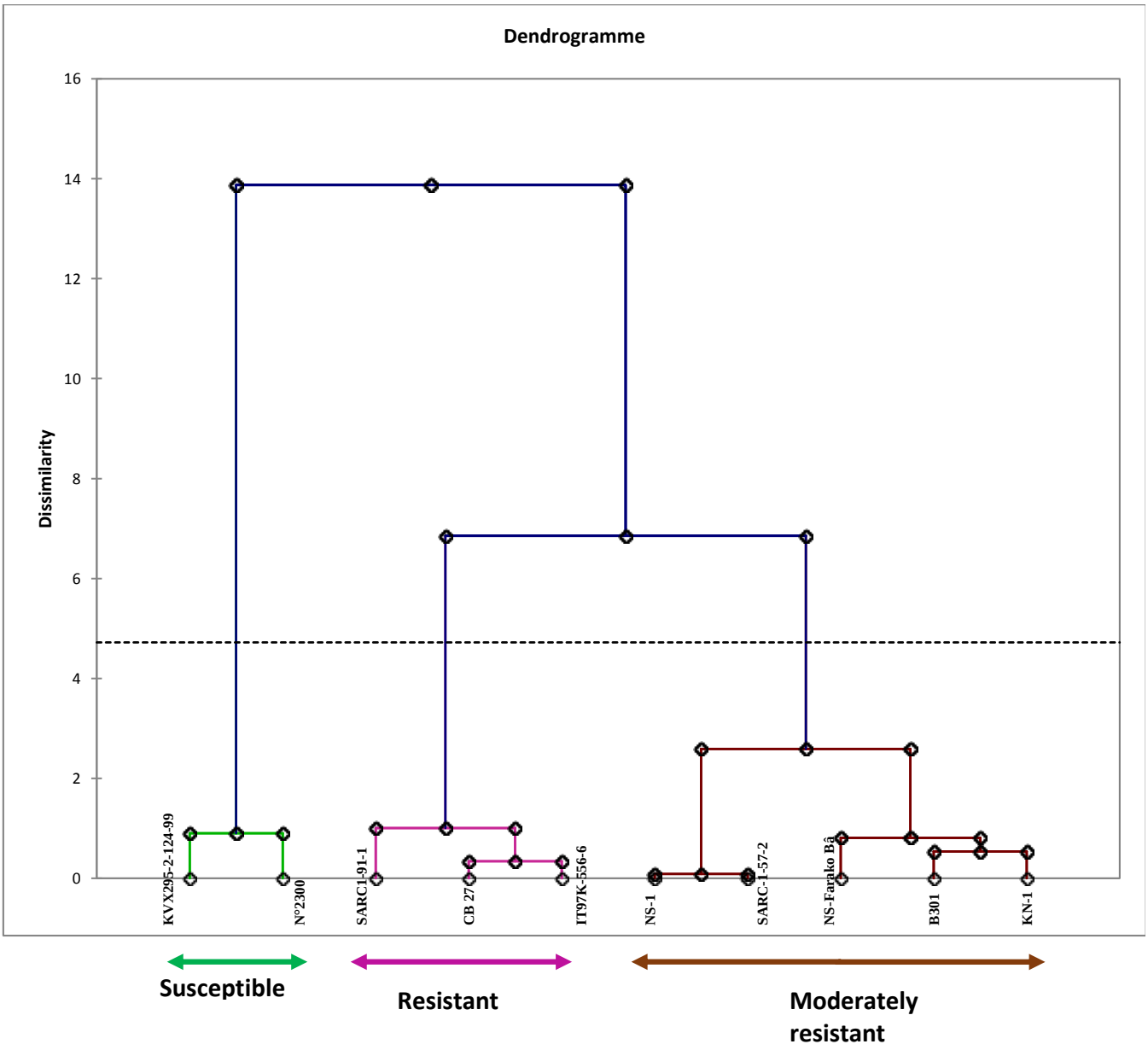

Figure 4. Dendrogram representing the three classes of lines (Strain of Bobo-Dioulasso).

this antibiosis activity often results in increased mortality or reduced longevity and reproduction of the insect.

The difference among aphids strains was highly significant (P = 0.007) suggesting that there is more than one aphids biotypes in Burkina Faso. The number of leaves being positively correlated with the survival rate and negatively correlated with the degree of infestation, these two variables were the best suited to classify the lines. For instance, the most resistant lines are those with the highest survival rate and the lowest degree of infestation, and the most susceptible lines are those with the lowest survival rate and the highest degree of infestation.

The summarized results of the clustering is showing that IT97K-556-6, SARC1-91-1, KVX295-2-124- 99 and KN-1 are resistant to aphids; B301, CB27, and SARC-1-57-2 are moderately resistant while N°2300 NS-1 NS-Farako Bâ are susceptible. These results are consistent with those of Kusi et al. (2010) who identified SARC-1-57-2 and SARC1-91-1 as resistant to aphids. IT97K-556-6 is the most resistant line to all the three strains of aphids with an average survival rate equal to 93.33% and a degree of infestation equal to 1.4. However, it was identified as moderately resistant by Souleyman et al. (2013). This nuance could be explained by the difference in aphid strains used or differences in the climate of the

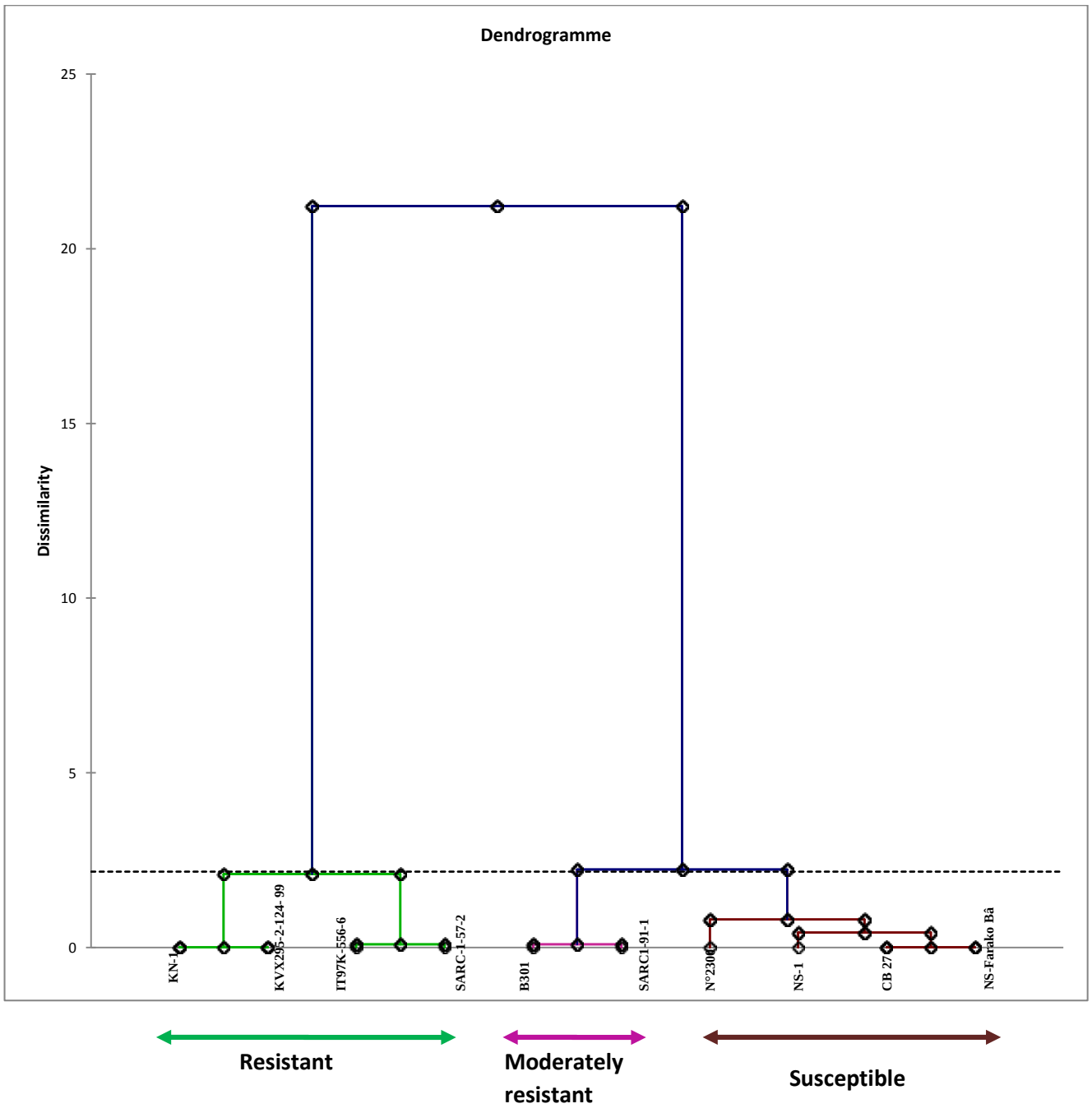

Figure 5. Dendrogram representing the three classes of lines (Strain of Pobé-Mengao).

two study areas. N°2300 is the most susceptible. It was susceptible to all the three strains of aphids with an average survival rate equal to 33.33% and a degree of infestation equal to 3.6.

According to IITA-SAFGRAD (1981) KN1 is adapted to different agro-climatic zones of Burkina Faso, but not resistant to cowpea insects. The present study showed that this line is resistant to aphids, the difference might be explained by a change in the aphids biotype used. The biotype 'K' or aphid's biotype of Kamboinsé was used for the screening (IITA-SAFGRAD, 1981). It may also be due to a physiological or even genetic change in the line. Moreover, in this study the confinement of aphids increased the parasitic pressure on plants. This pressure would have been able to induce an excessive compensatory response (Kusi, 2010) in KN1 physiological response, which was manifested by a much greater production of leaves. This compensatory response,

Table 4. Lines status.

Lines	Resistant	Moderately resistant	Susceptible
	IT97K-556-6	B301	N°2300
Lines	SARC1-91-1	CB 27	NS-1
	KVX295-2-124- 99	SARC-1-57-2	NS-Farako Bâ
	KN-1		

combined with the antibiosis activity developed by the line, gave it an unexpected resistance to aphids.

Conclusion

Cowpeas are of vital importance to the livelihood of several millions of people in Africa where it provide food and cash income. However, high yields in cowpea are seriously militated by insect pests like *Aphis craccivora*. Host plant resistance is an efficient and environmentally friendly way of controlling them.

The present study has shown that the speed of aphids' multiplication depends upon the genotype on which they develop. So the line IT97K-556-6 has exhibited the highest level of antibiosis. The screening test showed significant differences among lines. It was possible to identify four resistant lines (IT97K-556-6, SARC1-91-1, KVX295-2-124-99 and KN-1) and three moderately resistant (B301, CB27, and SARC-1-57-2) which may be used in breeding program to develop resistant lines or improve existing lines that are susceptible to *A. craccivora*. The study also showed significant differences among aphids' strains suggesting that there are more than one aphids' biotypes in Burkina Faso.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

Authors gratefully appreciate the Alliance for Green Revolution in Africa (AGRA) for sponsoring this work. We also thank Dr Patrice Savadogo and Pr J. N. Ayertey for reading and correcting this document. We are thankful to all the technicians at the laboratories of Genetics and Entomology at INERA.

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