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Full Length Research Paper

The impact of animals on crop yields in Malawian rural villages

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It is essential that the net effect of biodiversity on crop yields is determined; particularly in developing nations, where both increasing food security and reducing biodiversity losses are of high importance. This study modelled the abundance of pests, pollinators and pest-control animals and determined their impact on crop yield within agroecosystems in four rural villages in Malawi. Data on the habitat area, survivorship, fecundity, birthing month and effect on crop yield for 14 animal functional groups were collated through a focused meta-analysis. Using this data, models were created to determine the abundance of each functional group using land cover as the sole input variable; with Participatory Rural Appraisals (PRA) utilised to validate the model prediction. Bees, birds and insects always improved crop yield, whereas monkeys, rodents and large herbivores always result in losses. Three out of four villages experienced a net benefit to crop yield from the animal biodiversity present. We conclude that models derived from meta-analyses appear useful for broadly predicting the local-scale abundance of functional groups and their qualitative impact on crop yield. However, long-term field observations should be conducted to ensure that the PRA values in this study correlate with direct observation.

Key words: Africa, Malawi, Zomba, agriculture, pest, pest control, pollination, pollinator.

INTRODUCTION

Since the publication of the Millennium Ecosystem Assessment in 2005, ecosystem services (ES) have become an important topic in both policy and science (de Groot et al., 2009). Whilst policy is mainly concerned with provisioning services which contribute directly to livelihoods, such as food, water and timber, research has also focused on regulating and cultural services (Zhang et al., 2007). It is widely recognised that biodiversity underpins many ecosystem services (Mace et al., 2012) and thus is essential for human well-being. For example, biodiversity is vital in providing regulating services such as pollination and pest-control (Altieri, 1999; Cardinale et al., 2012) which are essential for food security (Ghazoul, 2005), particularly in developing countries which are typically biodiversity rich but technology poor. Pollinators, defined here as animals that transfer pollen between plants, are known to benefit agricultural through increasing crop yields, improving fruit and seed health and reducing crop deformities (Garibaldi et al., 2013;

Ricketts et al., 2004). Awareness of the importance of pollination for food security has risen and features in the policies of numerous governments (Byrne and Fitzpatrick, 2009). However, ecosystems can also provide disservices (Dunn, 2010). In agroecosystems, a major ecosystem disservice is pests causing agricultural yield losses (Cardinale et al., 2012). The term 'pest' is limited in this investigation to cover animals that consume crops from the field to market or consumption, and does not extend to bacteria or parasites (Oerke, 2006). Both vertebrate and invertebrate pests result in 14% of agricultural losses annually (Cerkal et al., 2009), having high economic losses and impacting food security (Oerke, 2006). Pest-control, defined here as the natural enemies of pests (so does not include use of agrochemicals), has received less attention within the ES literature (Chaplin-Kramer et al., 2011). Pest-control is becoming increasingly studied in agricultural systems to reduce pesticide use (Chaplin-Kramer et al., 2011; Mahr et al., 2001). However, pets-control studies typically focus on the use of exotic species, often to control invasive species rather than on the value of indigenous biodiversity to support crop production.

Whilst the interaction between pests and pest-control and has been studied (Chaplin-Kramer et al., 2011), the interaction between pests, pollinators and pest-control needs to be evaluated to establish the overall impact of ecosystem services provided by wildlife on agricultural systems, (Lundin et al., 2013). Currently, there is only one paper within the literature that focuses on this three way interaction and its impact on yield – the interaction of three species; bumblebees, *Apion* weevils and parasitic wasps, conducted in in laboratory conditions (Lundin et al., 2013). However, we have been unable to find a study that has investigated the interaction between pests, pollinators and pest-control in the field, nor linked such interactions to crop yields.

Where there is limited time and/or funds for field observations, existing literature values can be analysed through a meta-analysis (Bengtsson et al., 2005; Jonsen et al., 2003). These values, along with land-cover maps, can be used to predict animals present within a landscape and estimate their abundance. However, it is vital to validate these relatively simple, lookup table-based models to ensure they are accurate. Model validation can prove difficult in data-deficient regions, such as sub-Saharan Africa (Rodger et al., 2004), but it is such regions that are generally the most food insecure and thus show the greatest need for models that may help to increase crop yields (Rodger et al., 2004). Direct observation can only provide evidence of animal abundances for the period of fieldwork, which may be limited in many developing nations due to time and costs limitations and thus would give a very unreliable estimate of annual abundance and little indication of seasonal or annual fluctuations. Participatory Rural Appraisal (PRA) can be used to rapidly ascertain biodiversity within rural landscapes by using local knowledge (Hellier et al., 1999) and can contribute to wider understanding of ecosystems services in developing countries. Thus, enabling rural communities to provide abundance estimates via PRAs, a method frequently used in agroecosystem analysis, can fill the data void and be utilised to validate a model produced via a meta-analysis (Chambers, 1994).

Here, we produce a model though a meta-analysis that predicts the abundance of animal species within four villages in Malawi, sub-Saharan Africa. We then use this model to predict the net crop yield benefits or losses that the villages incur from the amounts of the pests, pollinators and pest-control present. We then validate the models using PRAs conducted in the same four villages. To the best of our knowledge, ours is the first study combining models of the effect of pests, pollinators and pest-control on crop yield within sub-Saharan Africa, one of the worlds poorest and more food insecure regions.

METHODS

Model creation

Meta-analysis

Articles in peer-reviewed journals, books, organisational reports and governmental reports (identified using Google scholar, Jstor and Web of Knowledge and specific search terms (Appendix 1, Table 1)) were utilised to compile data on the area required for each animal functional groups (FG) to live in (habitat area), the chance of an individual surviving a month (survivorship), the number of offspring an individual produces annually (fecundity), the time of year the individual gives birth (birthing month) and the effect on crop yield, recorded as a percentage increase/decrease. FGs were chosen because the literature did not contain adequate species-specific data. After consultation with local experts in Malawi, ten FGs were selected: amphibians, bees, birds, other flying insects, ground insects, rodents, large herbivores, bats, monkeys, and reptiles. These models were further divided into their respective ecosystem service and disservice functional role: pollinator, pest, and pest-control. For example, the 'other flying insects' FG contained families such as grasshoppers that only act as pests; however, ants act as a pests, pest-control agents and pollinators. Consequently, the characteristics of the 'other flying insects' pest FG differed from that of pollinator and pest-control. This further subdivision resulted in 14 FGs (Figure legend of Figure 1 and Appendix 1). The values collated from the literature were used to calculate the mean and 95% confidence intervals for the area required for each land-cover the FG lives requires (habitat area), fecundity, survivorship and effect on crop yield for each FG. In some instances, habitat area of a FG for a specific land-cover could not be obtained. When this arose, the mean habitat area required was applied to all land-covers that the FG was documented within the literature as occupying.

Application of meta-analysis to predicting animal abundances at the village scale in Malawi

We used the values from the literature to predict abundance at the scale of the village for four Malawian villages, three in the Zomba District and one in Machinga District. The villages utilised were selected by ESPA-ASSETS (http://espa-assets.org) and fall on a transect between Zomba Plateau and Lake Chilwa.

Land-cover for each village was derived from two land-cover maps, a standardised African land-cover map (Roger et al., 2013) and Globcover2009 (European Space Agency, 2011). As

Table 1. The net impact of functional groups on crop yield determined from the meta-analysis.

Functional group	Effect on yield (mean)	Effect on yield (Lower 95% CI)	Effect on yield (upper 95% CI)
Pollinators			
Bees	72.44	65.18	79.71
Birds	460.50	-418.75	1339.74
Other Flying Insects	106.00	106.00	106.00
Pests			
Other Flying Insects	-44.34	-53.85	-34.84
Ground Insects	9.39	-5.03	23.81
Rodents	-26.40	-36.82	-15.97
Large herbivores	-39.11	-46.54	-31.69
Pest-control			
Amphibians	0.00*	0.00*	0.00*
Bats	250.26	233.35	267.16
Birds	-14.18	-32.89	4.52
Ground Insects	35.03	32.08	37.97
Monkeys	-80.90	-88.03	-73.77
Other Flying Insects	24.35	16.52	32.17
Reptiles	0.00*	0.00*	0.00*

*zero values are due to a lack of literature on the functional group's impact on yield.

the model abundance predictions were not sensitive to the land-cover map used (Appendix 2), results in this paper will be presented for the Globcover2000 map only as this map was deemed the most accurate following on-the-ground validation.

Predicting animal abundances

R v.3.0.1 was then used to build the models (R Development Core Team, 2010). Abundances were predicted using land-cover as the solitary input. The starting numbers and the carrying capacity were determined by dividing the available habitat by the area required for each FG. The model ran using monthly time-steps. Each month the FG populations would decline due to death, with the probability of survival matching the value determined by the meta-analysis. Due to a lack of data concerning when species are most likely to die in the year, death was assumed to act linearly throughout the year. Populations also increased in monthly increments due to fecundity. However, this did not occur linearly as there was sufficient literature on birthing months. Thus, the annual fecundity was split into monthly values, weighted by the proportion each month was identified within the literature as being a birthing month. Furthermore, births only arose if the FGs population did not exceed the carrying capacity, also determined from the meta-analysis. Consequently, seasonal variation is modelled through variation in fecundity and not survivorship.

In total, three models were built for each FG: one parameterised by using the means from the meta-analysis and two parameterised using the upper and lower confidence intervals respectively. Each model was run for all four villages.

The models were run between 1977 and 2013, but a pre-run period from 1800 was allowed to ensure that the models had stabilised by the time period of interest.

Model validation

Participatory rural appraisals (PRA)

The PRA for this study were conducted with a spread of ages, socioeconomic status and geographic spread throughout the village (for a complete version of the PRA questionnaire see Appendix 1). PRA groups were separated by gender to ensure that socioeconomic biases and traditions did not affect participation and so all members felt they could participate freely. At each village, two male and two female PRA were conducted. The PRA were conducted in the local language spoken by the participants (a mixture of Chichewa and Yao) using images of example species for each FG type (Appendix 1). Primarily, data were collected on how many individuals they saw within each FG in the village. Present day numbers were reported as well as recall of 10 years ago, 20 years ago and 30 years ago. Where village-wide estimates were not practicable, individuals were asked how many they would see in a unit of known area or time that they felt able to comprehend, e.g. a field/house or observations per day. For the purpose of the PRA, FGs were further subdivided (Appendix 1) to make numbers easier to report as more specific groups were discussed at a time. For example, large herbivores were split into small antelope, large antelope, elephant and hippopotamus. In total, 28 groups were used in the PRA (Appendix 1). Additional information such as the land-cover in which the FG resides, its benefits and disadvantages and how the FG is controlled were also recorded (Appendix 1).

Data preparation and analysis

The PRA participants typically reported animal abundances in four ways: 1) the number of individuals throughout the whole village; 2) the number of individuals in a house; 3) the number of individuals in a field; and 4) the number of individuals seen in a day. Thus, before comparing the PRA values to the models, all PRA estimates needed to be post-processed, scaling all estimates up to the village area. For numbers reported per house, the count given was multiplied by the number of households for the respective village as it was assumed that the FG was present in all houses. The household values were taken from the Malawi Spatial Data Portal (MASDAP, 2013).

Specific data was not available for the number of fields in each of the four villages. Consequently, the average field size for the respective districts of the villages were taken from the Malawian National Census of Agriculture and Livestock
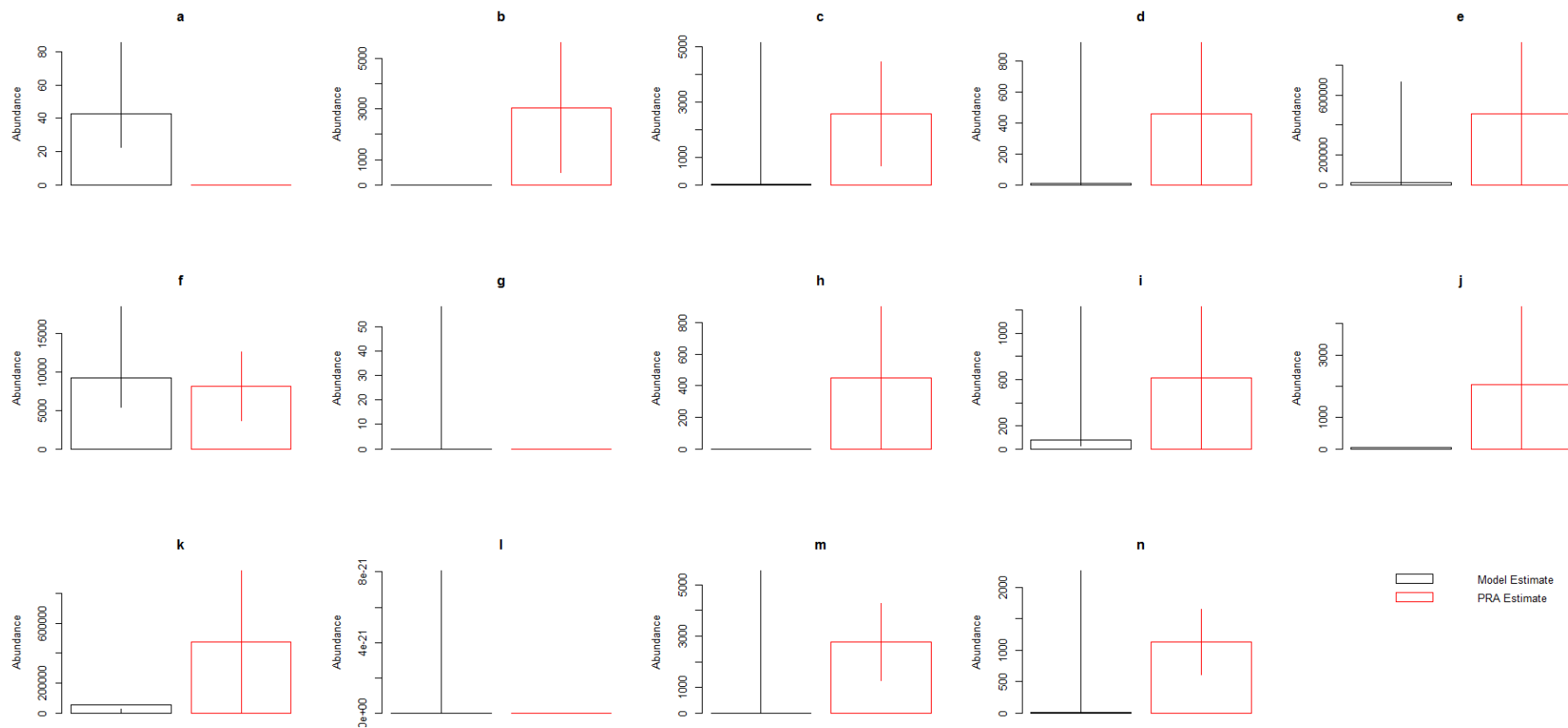
Figure 1. Model and PRA predicted abundances in Village A of: a) Pollinating bees; b) Pollinating birds; c) Pollinating other flying insects; d) Other flying insect pests; e) Ground insect pests (also pollinators); f) Rodent pests; g) Large mammalian herbivore pests; h) Amphibians that control pests; i) Bats (pollinators, pests and pest-control); j) Birds (pests and pest-control); k) Ground insects that control pests; l) Monkeys (pests and pest-control); m) Other flying insects that control pests; n) Reptiles that control pests.

2006/2007 Main Report, 0.264 ha and 0.309 for the Zomba and Machinga districts respectively (National Statistical Office, 2010). The area of cropland within each village was determined using the Globcover2000 land-cover map and divided by the mean field size of the appropriate district to give the number of fields within the village. This number was then multiplied by the count of individuals observed to give the village total. Finally, for some estimates, participants could only report the number of individuals they see in a day. This temporal estimate was converted to a unit area using the following method. The average sub-Saharan African villager walks 5 km a day (Schafer, 2000), combining this value with the assumption that an individual can see 10m either side of themselves means a villager observes an area of 0.1 km^2 a day. It was considered unlikely that villagers would be able to travel freely over large water bodies and thus water bodies were deducted from total village area before the abundance estimate provided for 0.1 km^2 could then be scaled up to the village area. The more specific functional groups for the PRA exercises were then regrouped according to the meta-analysis FG (Appendix 1). These PRA estimates were analysed to give the mean and

95% confidence intervals of FG populations in each village.

Calculating the net effect of FGs on crop yield

The impact of FGs on crop yield was also estimated through the meta-analysis. In most instances, the direct effect on yield was available for the pest and pollinator FGs. However, data on the effect of pest-control FG on crop yield were sparse. Consequently, to determine the impact of many pest-control FGs, the impact of pest-control presence on pest abundance was estimated from the literature and the reduction of crop damage that arose from this was attributed to the pest-control FG. Thus, a lookup table of impact on crop yield was produced, using R v.3.0.1, with the mean and 95% confidence intervals impact on crop yield for each FG (R Development Core Team, 2010).

The net impact of the FGs on crop yield in each village was determined by combining a presence/absence rule with the newly created look-up table. Therefore, if a FG was indicated as present, then its associated impact on crop yield would be summed to that of all other FGs present to obtain the net impact of biodiversity on the crop yield at a village scale. Presence or absence was used rather than multiplying by the number of individuals present due to data deficiency on the impact of individual animals on crop yields. This procedure was repeated for the upper and lower confidence intervals to determine the likely yield impact a village may experience from the FGs present.

RESULTS

Animal abundance

PRA values fell within the confidence intervals for 71.4 to 85.7% of the FG abundances predicted by the models (85.7, 78.6, 71.4 and 78.6% for village A, B, C and D respectively). Thus, the models predict perceived animal abundance across the region surveyed relatively well.

Specifically, abundance values from the PRA exercise consistently fell within the confidence intervals of the models produced by the meta-analysis when predicting the abundance of insects (pollinating other flying insects, other flying insects that act as pests, pollinating and pest ground insects and, pest-control other flying insects; a, d,e and m respectively of Figures 1 to 4), as well as for birds that act as pests and pest-control, monkeys and reptiles (j, l, and n of Figures 1 to 4) across all four villages. The mean PRA abundance values for all other flying insects categories, as well as pest and pest-control birds and reptiles were higher than the model mean for all four villages (c, d, e, j, and n of Figures 1 to 4). For monkeys, the PRA and model mean are the same for villages A and B (mean = 0). However, for villages C and D the PRAs had higher abundance means than the model (0 vs. 508 and 0 vs. 90 respectively). Conversely, ground insects that act as both pollinators and pests had lower abundances reported in the PRAs than the models predicted in villages B, C and D. Village A PRAs reported higher mean abundances of ground insects that act as both pollinators and pests (m of Figures 1 to 4).

Other model results showed less consistency across villages. Models for four other FGs predicted values that the PRA confidence intervals fell within for three out of four villages (pollinating birds, rodents, large herbivores and, bats (b, f, g, and i of Figures 1 to 4 respectively)). The PRA abundances for Village A were higher than those predicted by the model for pollinating birds (3045 ± 2,558 and 0 individuals respectively (b of Figures 1 to 4)). The PRA abundances were also higher than the model predictions for bats (15,540 ± 14,282 and 36-5,689 individuals respectively) and large herbivores (0-84 and 0 individuals) in village C. In village B, reported PRA abundances for rodents were lower than the values predicted by the model (17,581 ± 11,767 and 38,289-405,588 individuals respectively). Furthermore, the models predicted a greater abundance of bees and pest-control ground insects compared to the values given in the PRA exercises (a and k of Figures 1 to 4) across three out of four villages. For Village A, the PRA value for pest-control ground insects is higher than the value predicted by the model (0-1,406,345 and 1,212-55,112 individuals respectively. The model predicted bee abundance in village C close to the values reported in the PRAs (34-5,362 and 0-217 with mean of 68 and 80 individuals respectively). Finally, in villages C and D the model predicted that there were no amphibians present. However, the mean PRA values for amphibians were 325 and 406 individuals, respectively for these villages and the lower confidence intervals are both above 250 individuals. The mean PRA abundances for villages A and B were higher than the model mean (450 and 84,205 respectively compared to 0). However, the lower confidence interval for the PRA values was 0 for both villages. Consequently, the model prediction fell within the PRA confidence intervals for villages A and B.

Whilst the estimated animal abundance derived from the models was mostly accurate, the variation in this abundance throughout the year (i.e. between seasons) did not match that reported in the PRA exercise (Appendix 2). Furthermore, change through time predicted by the model was not representative of the values given in the PRAs (Appendix 2).

Effect on yield

Functional groups

Overall, the net effect of bees, bats, other flying insects and ground insects on crop yield were significantly positive once all ecosystem services (pollination and pest-control) and disservices (pests) were taken into account (Table 1). Conversely, the net effect of rodents, large herbivores and monkeys on crop yield were significantly negative (Table 1). Finally, the confidence intervals for pollinating, pest and pest-control birds overlapped zero and so it cannot be determined from this study whether birds increase or reduce yield (Table 1). Participants did not perceive and/or could not report any yield increases caused by FGs. However, the perceived damage was often
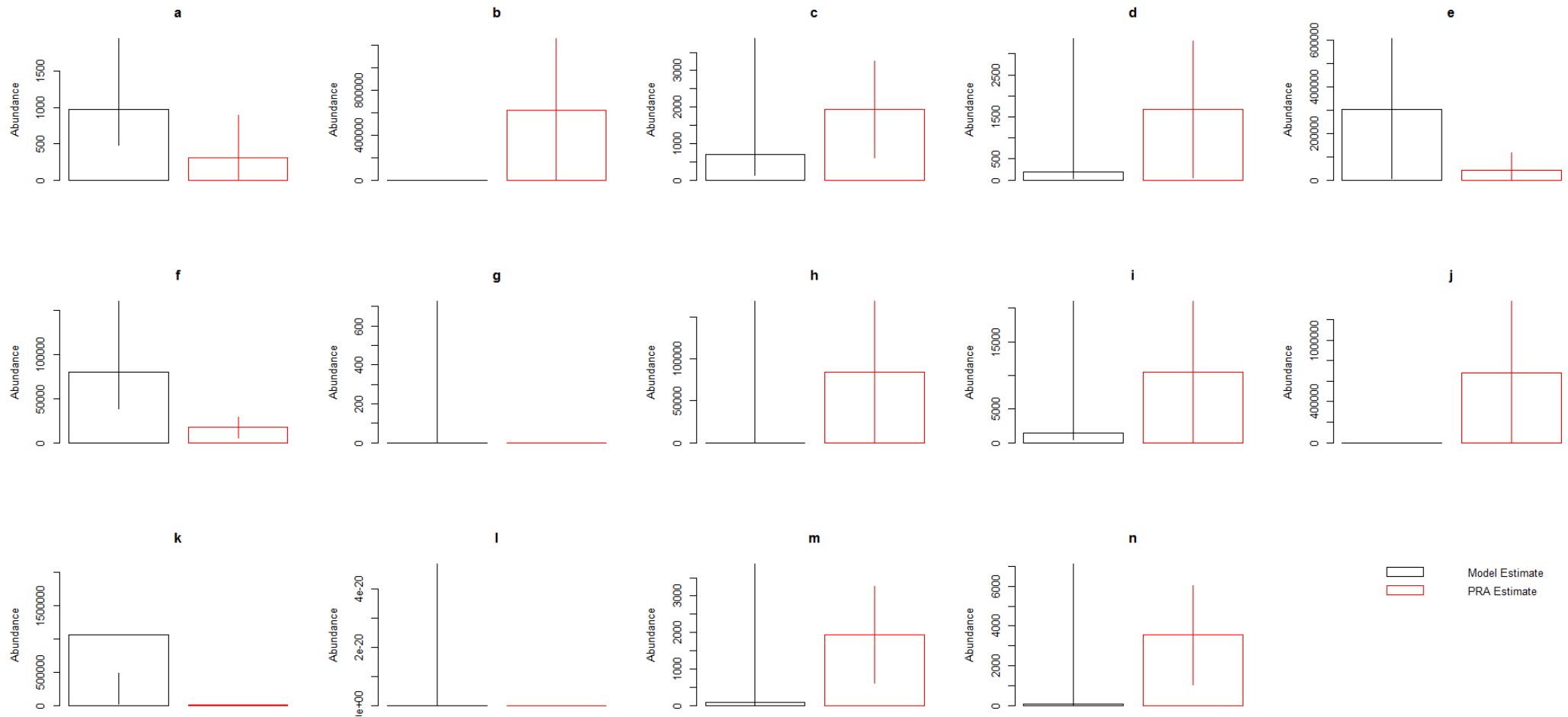
Figure 2. Model and PRA predicted abundances of FGs in Village B. FG codes are as listed in Figure 1.

greater than the values reported from the meta-analysis, with the exception of monkeys, which was lower (Table 2).

The cumulative effect of FG on crop yield

Overall, both the models and the PRAs suggest that

the net effect of animal biodiversity in villages C and D resulted in a net benefit to crop yield overall for both the model and PRA values (Table 3). The confidence
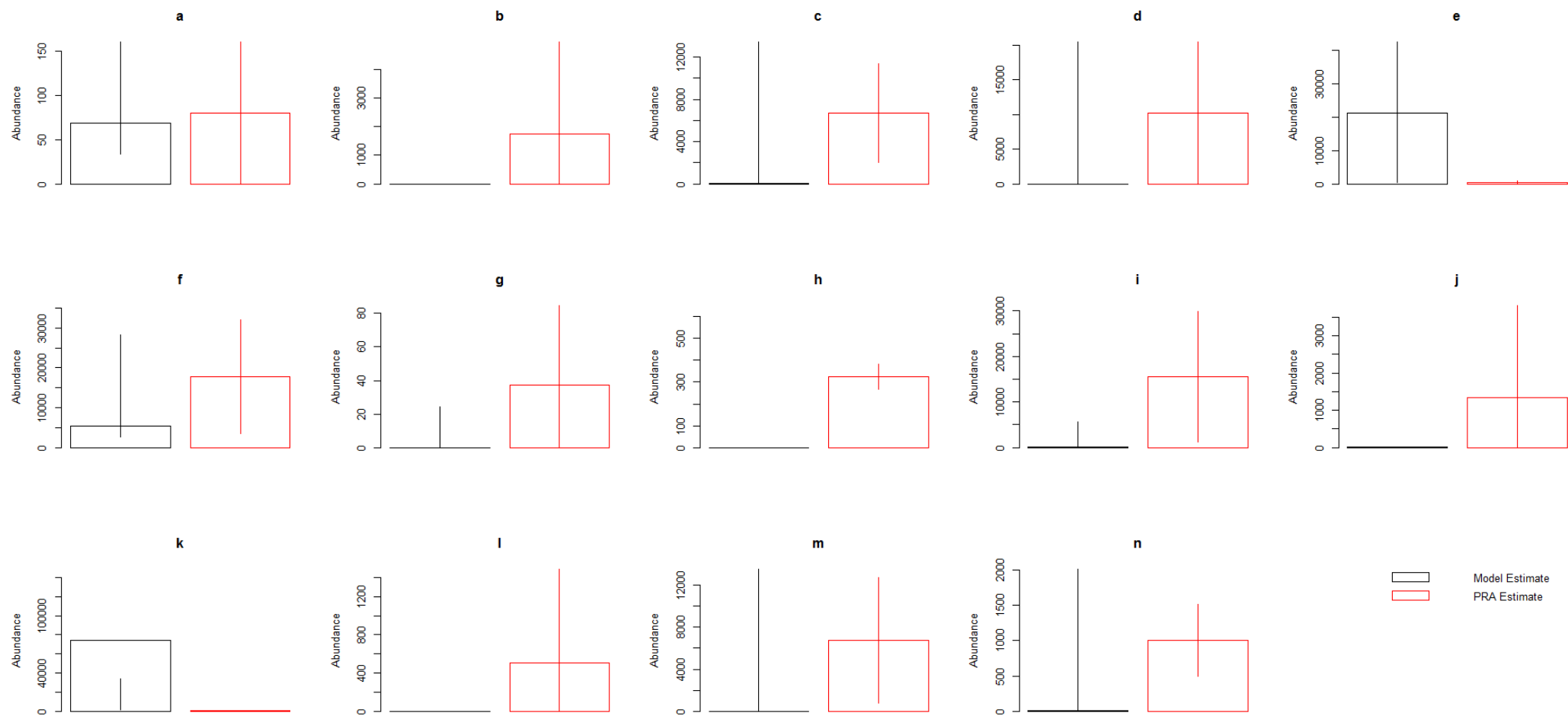
Figure 3. Model and PRA predicted abundances of FGs in Village C. FG codes are as listed in Figure 1.

intervals for village B cross zero for the values obtained by the model. Consequently, the model is unable to predict whether the village experiences an increase or reduction in crop yield due to the

interaction of the FGs present. This is contrast to the PRA, which indicates that the FGs present in village B have a positive impact on crop yield. Similarly, the model and PRA estimates are not in agreement for

village A. The model predicts that village A experiences a net increase in crop yield, however, the PRA confidence intervals overlap zero and therefore suggest that it cannot be determined whether or not
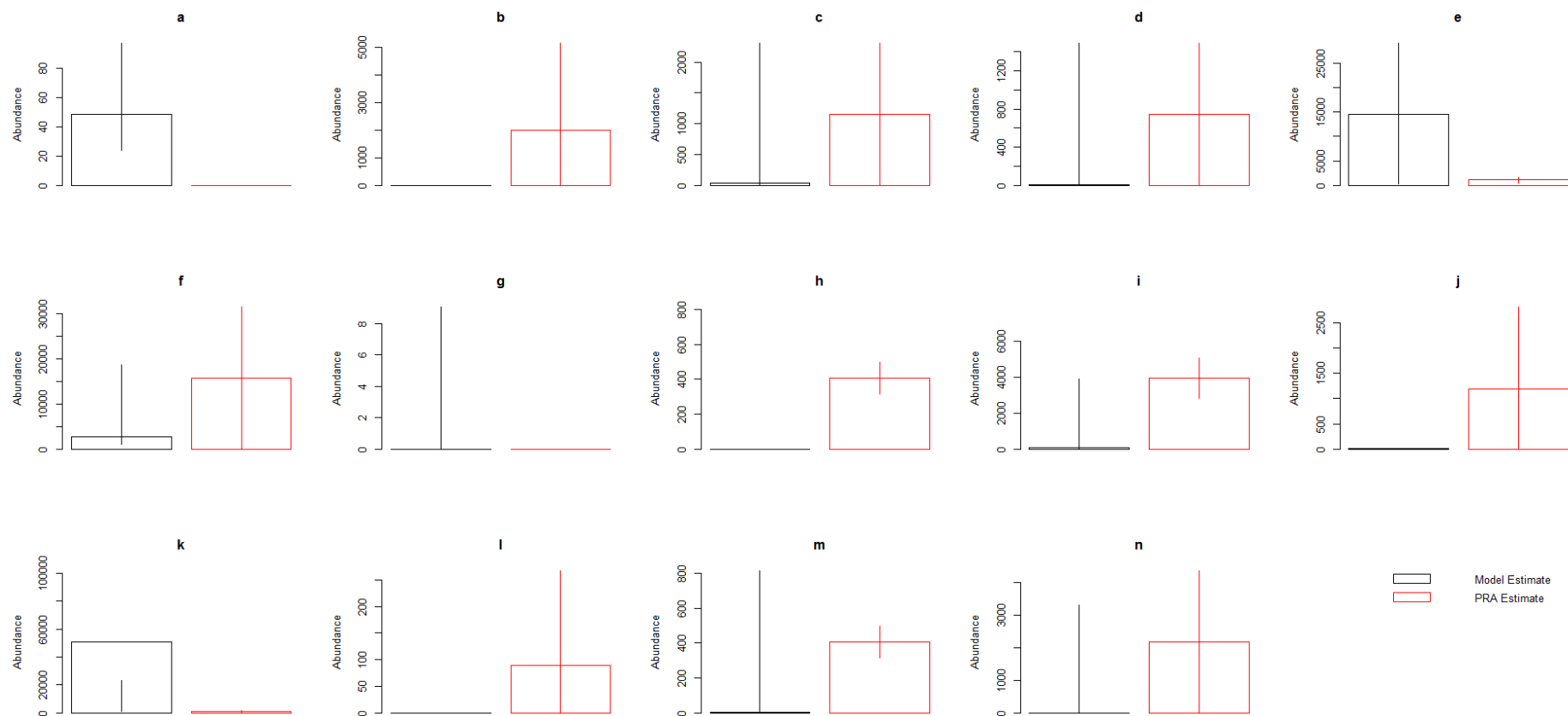
Figure 4. Model and PRA predicted abundances of FGs in Village D. FG codes are as listed in Figure 1.

there is a gain or reduction in yield due to the interaction of the FGs present.

DISCUSSION

The net impact of biodiversity on crop yield

Our results: The first to quantify the cumulative effects of pests, pollinators and pest control agents in a natural system indicate that the net effect of animal biodiversity present within in rural southern Malawi is beneficial to crop yield. This is because although pests can result in large losses in yields, such losses are more than offset by the positive effects of pest-control and pollinator FGs. Our result support the laboratory-based findings, which show that interactions between pollination and pest-control is beneficial to crop health (Lundin et al., 2013), whilst those between pest and pest-control can increase yields (Chaplin-Kramer et al., 2011).

While not based on primary field data, subsets of our meta-analysis and PRA-based analysis also correspond to field-based studies, increasing our confidence in our results. For example, our study, like numerous others, shows that bees were always beneficial to crop yield (Hoehn et al., 2008; Ricketts

Table 2. The impact of functional groups on crop yield determined from the PRAs.

Variable	Birds	Rodents	Monkeys	Large herbivores	Ground insects	other flying insects	Bats
Crop damage (%)	-45.28	-43.8	-58.48	-61.00	-71.09	-67.88	-5.00

Table 3. The net effect of the functional groups present on crop yield in each village- model and PRA outputs.

Variables	Village A	Village B	Village C	Village D
Model mean	350.90	811.40	350.90	350.90
Model lower	246.38	-155.85	262.90	246.38
Model upper	1746.96	1746.96	1746.96	407.22
PRA mean	738.95	811.40	691.39	658.05
PRA lower	-333.05	2.30	289.49	215.28
PRA upper	1698.93	1778.65	1673.19	1625.16

et al., 2004). Additionally, in agreement with previous studies, our results show that rodents (Oerke, 2006; Sánchez-Cordero and Martínez-Meyer, 2000; Stenseth et al., 2003) and monkeys (Wallace and Hill, 2012) result in crop yield losses. Our study also found that ground insects and other flying insects had a net benefit for crop yield. Whilst it is well-recognised that many species within this FG such as wasps, flies, ants and beetles act as pest-control (Lundin et al., 2013; Mahr et al., 2001; Way and Khoo, 1992), the literature has traditionally focused on the crop yield loses that result from the damage these FGs cause (Adams et al., 1983; Motzke et al., 2013; Oerke, 2006; Wood et al., 1980). However, conflicting studies have found that other types of ants, beetles, flies and wasps, are beneficial to crop yield (Garibaldi et al., 2011; Garibaldi et al., 2013; Lundin et al., 2013).

Our meta-analysis revealed important limitations and biases within the literature on pests, pollinators and pest-control agents. Firstly, much of the literature surrounding the yield impacts of biodiversity focuses on qualitative changes as opposed to quantitative measures and so could not be included in the meta-analysis. Whilst numerous studies state whether a FG caused a reduction or an increase in yield, few put a definitive value on the impact (Garibaldi et al., 2013; Reitz, 2009). Secondly, there is a bias within the scientific literature towards crop losses caused by FGs (21 references giving 122 values) but those that benefit crop yield are more rarely quantified (8 references for both pollination and pest-control, giving 14 values and 32 values respectively). Furthermore, the studies that quantified beneficial impacts on agriculture often did not directly investigate this. For example, only two of the 32 pest-control values, one for birds and one for bats, were directly reported as the impact of pest-control on yield (Kalka et al., 2008). The remaining values had to be indirectly inferred, for example by calculating the crop damage reduction resulting from the consumption of pests. Within the pollination literature there is a distinct bias towards the impact of bees, with many other functional groups only discussed by a single reference, despite the fact that other species are as beneficial to pollination as bees (Garibaldi et al., 2013).

Interestingly, the biases of the local communities in their perception of the impact of animal biodiversity reflect the skewed research focus on crop loses. Overall, perceptions of impact were largely negative with only a small number of positive impacts perceived. When PRA participants were prompted about benefits such as pollination they frequently possessed no knowledge of the process. Within each village, there would typically be one or two young male participants that knew of the term pollination but, when asked of its impacts, they did not think it changed yields but that it created hybrid varieties. In addition, participants infrequently recognised that some FGs acted as pest-control. Participants rarely knew what animals preyed upon and, if they did, it would be because the prey had a direct effect on their wellbeing (such as lizards and bats eating mosquitoes, which directly cause malaria) rather than an indirect effect (e.g. agricultural pest-control reducing pest numbers which in turn increases yield and increases wellbeing). The lack of pollination and pest-control observations by participants may be due to these services being too subtle for participants to observe and, even when observed, too difficult to quantify and thus appreciate. The inability to perceive these subtle relationships led to some substantial differences between the perceived impacts and those known to science (Hellier et al., 1999). For example, some participants thought that ladybirds gave birth to aphids thus, they assisted in causing crop damage rather than recognising that ladybirds were found with aphids as they consume them (Snyder et al., 2004).

In addition to services not being observed, disservices were overestimated by participants. This finding is in agreement with previous studies which also found that participants perceived greater yield losses than actually occurred (de Mey et al., 2012). However, in some instances, participants overlooked negative effects. For example, some participants did not view fruit being eaten by bats as an issue as the bats primarily fed on the over-ripe bananas that they did not intend to sell. Participants also perceived

monkeys as less damaging than the model predicted. However, this is due to the meta-analysis value being for baboons when the majority of monkeys present within the villages were smaller monkey species. Thus, participants did not necessarily perceive less damage than the model. The values were simply less comparable than other FGs.

An important limitation of this study is that it only encompasses the net impact of animal biodiversity on crop yield and not the overall net effect of all ecosystem services and disservices provided by animal biodiversity. This is because we assumed that the most important ES impact of biodiversity in these agriculture-dominated villages with major food security issues would be on crop yield. Intriguingly, qualitative observations we made while conducting the PRA suggest that this may not be the case, and that the most important ES impact of some FGs is on cultural and not provisioning services. For example, participants often did not perceive groups such as snakes, which provide pest-control services, as beneficial. Despite knowing that snakes consume rodents (which cause large crop losses), participants frequently stated that they would rather kill a snake than allow it to survive, as some snakes have the potential to kill people. A similar situation arose with bees. Participants would frequently report killing bees, despite the fact they provide honey and pollination services, because they can be a danger to people. However, as previously discussed, participants may be more inclined to arrive at this decision as they often did not perceive the pollination benefits of bees. Conversely, FGs that resulted in large losses such as monkeys and birds frequently had positive cultural services, such as providing entertainment, keeping people happy and being aesthetically pleasing. Previous studies have found that participants usually value provisioning services more highly than cultural services (Pereira et al., 2005), but it is evident from this study that cultural services can have a large impact of the tolerance communities may show to some pest species, even within subsistence agriculture dominated rural communities.

Using land-cover to predicting animal abundance

Previous studies have shown that meta-analyses can be used to predict animal abundances within conventional and organic agriculture (Jonsen et al., 2003). However, thus far, studies have focused on invertebrate abundances (Bengtsson et al., 2005; Jonsen et al., 2003; Langellotto and Denno, 2004). To the best of our knowledge, this is the first study to show suggest that a meta-analysis could be utilised to predict the presence and abundance of both vertebrate and invertebrate FGs in agroecosystems in Malawi. The models were most capable of predicting the abundance of pollinating, pest and pest-control other flying insects, pollinating and pest ground insects, pest and pest-control birds, monkeys, and reptiles. This can be partially attributed to wide confidence intervals of both the model and PRA.

This study showed that the models were generally not sensitive to the land-cover map utilised (Appendix 2). This is because the FGs within this study can be considered as predominantly generalists, occupying multiple land-covers. Thus, if one map labelled a particular area as woodland and another map identified it as forest, the FG abundance estimate could be unaffected if the group was equally likely to reside within both of these land-covers. This is important for future models as the models could be run in many locations, using many land-cover maps and still have comparable results. However, it is important to note that whilst the results from differing land-cover maps were similar for generalists, the resolution of the land-cover map had a substantial impact on the abundance predicted for specialised FGs. For example, the inability of the model to predict amphibians in villages C and D is due to the land-cover maps not containing water within these villages. However, the participants would frequently report frogs in the villages, as they would come from nearby rivers, which were too small to be observed on the land-cover maps.

Whilst the models predict annual animal abundance well, they were unable to capture the seasonal fluctuations that the participants perceived. For example, the meta-analysis suggests that the abundance of bees should fluctuate across the seasons, whereas the villages suggested that these populations were consistent all year round. This is perhaps because, due to data limitations, many studies included in the meta-analysis that addressed seasonal variation were from regions distant to the study site and thus derived from different seasonal regimes (Appendix 2). FGs that had African specific data matched perceived fluctuations the closest (e.g. birds; Appendix 2). This suggests that the models could be improved by obtaining Malawi specific birthing months, perhaps using local expert opinion as published data is lacking.

Previous work has argued that PRAs can be utilised to determine biodiversity within agricultural (Pereira et al., 2005) and tropical (Hellier et al., 1999) ecosystems. However, in accordance with this study, wide variations within the values obtained via PRAs have previously been reported (Pereira et al., 2005). Furthermore, PRAs are only able to provide estimates of perceived abundances and impacts. These perceived effects might not correlate highly with actual abundances or impacts. In fact, PRAs have been noted as providing over-estimates (de Mey et al., 2012). Providing over estimation is recognised and accounted for, PRAs may be utilised to collect historical data where records are not available and seasonal data where time is limited.

Study limitations

The main limitation of this study was data deficiency. Model validation through field observations was not possible as observations would have to be conducted

over a several years in order to obtain seasonal values as well as capturing yield variations. Consequently, PRAs were utilised as a rapid method to validate the models. Furthermore, there were data limitations within the scientific literature, which was depauperate of some values and thus unable to support more complex models. The meta-analysis and subsequent estimates could be further improved if this data were available. Additionally, it is recognised that there are relatively few studies from the direct study area, or even from Malawi as a whole. Future studies could use weighted means, giving more influence to values geographically nearer to the study area (Willcock et al., 2014; Willcock et al., 2012).

Validating the models using PRAs has limitations. As aforementioned, PRA participants often overestimate numbers (de Mey et al., 2012). Furthermore, perceived abundance estimates varied considerably for some FGs within a village (e.g. bats could range from 0 to 25,496) resulting in wide confidence intervals (Pereira et al., 2005). However, this uncertainty may have been due to the methods utilised to extrapolate some values to village level. For example, bats were reported as the number of individuals seen in a house thus this was multiplied by the number of houses within the respective village. It is highly likely that this resulted in an over estimate as bats were reported as preferentially occupying houses with tinned roofs. As the proportion of thatched roofed homes within a village was unknown, reported values were multiplied by all the houses in the village. Village A, which had the closest PRA and model means for bats, reported values as the amount seen in fields and so did not suffer from this bias. However, for this observation (and others like it), the number of fields in the village was calculated by dividing the area of cropland identified on the land-cover map by the average field size for the region, as reported by the National Statistics Office (2010). In truth, the average field size may have varied significantly between villages (National Statistics Office, 2010). Furthermore, when numbers within a unit area could not be provided, participants would state the number of individuals within a FG that they saw per unit time. Whilst this was converted to an area using the average distance travelled per day (see methods for full details), it was assumed that participants did not observe the same animal individual in multiple places. Furthermore, it was assumed that, within 10 m of the villager, all FGs are equally detectable. This is not the case, e.g. an antelope is obviously much more detectable than a beetle. The PRA values for ground pest and pest-control insects evidences this as they were often under predicted compared to the model mean as they are hard to observe. For example, thrips were frequently reported as not being present, despite casual observation by the authors. On one occasion, a thrip was pointed out to the participants who had recently denied the presence of thrips, causing them to remark that thrips which was too small for the participants to take note of.

The broadness of FGs whilst conducting the PRAs

also led to limitations. Participants were asked about small birds but pollinating birds were not separated from this group, as speaking about separate species compromised discussing broader FGs. Thus, model values for pollinating birds have been validated against perceived abundance of small birds and so should be expected to be lower than the PRA estimates. In some instances, numbers were not obtained at all for FGs, especially birds, rodents and insects as participants felt that they were "uncountable". To overcome this, fieldwork should be conducted to obtain abundances of species that participants perceive as "uncountable".

The models also have wide confidence intervals. This is due to the variation of species and families within each of the FGs. This variation resulted in a wide range of values recorded for habitat area within the model. For example, ants [*Formicidae*] in pollinating and pest-control insects ranged from requiring 3.33e-6 km^2 (Traniello, 1989) and 0.12 km^2 (Hölldobler, 1976). Previous models predicting insect abundance have narrower confidence intervals (Bengtsson et al., 2005; Jonsen et al., 2003). However, these models require more data inputs and so are perhaps less feasible in data-deficient regions such as Malawi. This study solely utilises land-cover to estimate animal abundance and other studies using a similar approach also report wide confidence intervals (Bender et al., 1998).

Conclusion

Overall, our results suggest that animal biodiversity with rural villages in Malawi results in an overall benefit to crop yields as the ecosystem services of pollination and pest-control outweigh the disservice of pests. Our work also highlights several biases within the literature, resulting in data-deficiencies when describing the quantitative impact of pollinators and pest-control on crop yield. This bias is shared by participants, as PRA participants often did not perceive pollination or pest-control services, focusing instead on the ecosystem disservice provided by pests. Finally, preliminary evidence indicating that participants prioritise some cultural values and other wellbeing increases over increases in crop yield is provided, indicating that commonly held assumptions about provisioning services being more important than cultural services may need re-examination.

Conflict of Interest

The authors have not declared any conflict of interest.

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Conflict of Interest

The authors have not declared any conflict of interest.

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Review

Physiological mechanisms of drought tolerance in sorghum, genetic basis and breeding methods: A review

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Sorghum is grown in semi-arid to arid regions of the world and serves as the staple food for about half a billion people in sub-Saharan Africa and Asia. The adaptation of grain sorghum to a wide range of environmental conditions has led to the evolution and existence of extensive genetic variation for drought tolerance. Consequently, sorghum is expected to play an increasingly important role in agriculture and meeting world food demand in the face of climate change, land degradation and increasing water scarcity. Drought is a complex phenomenon, and is considered one of the most significant factors limiting crop yields around the world and continues to be a challenge to plant breeders, despite many decades of research. Underestimating the genetics and the physiological mechanisms underlying drought tolerance is vital for the breeding to alleviate adverse effects of drought in order to boost productivity. In this literature review, research findings from the 1970s up to present are included. Most of the basic researches on the mechanism of drought tolerance were done in the early 1980s, and most of the current researches focus on verification and fine-tuning of methodologies. The paper outlines the main effects of drought on crop growth and development, and yield. It then examines the basic information on physiological mechanisms of drought in crops. Subsequent discussion is given on the genetic control of drought tolerance, and breeding methods in sorghum.

Key words: Moisture stress, drought tolerance, breeding, genetics, mechanisms, *Sorghum bicolor*.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench, [2n = 2x = 20] is the emerging model crop species for the tropical grasses with C4 photosynthesis. Sorghum is the fifth most important Cereal crop and occupies the second position among the staple food grains in semi-arid tropics. It remains a critical component of food security for more than 300 million

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in Africa. Sorghum serves as a dietary staple crop for millions of people, especially in arid and semi-arid farming systems. Additionally, sorghum grain is used as livestock feed and for production of local beverages, while the stalk is used for animal feed, firewood, and as a construction material (McGuire, 2000). Sorghum grows across a wide geographic area at various altitude, day length, rainfall, and temperature regimes. Consequently, it is well adapted to withstand harsh conditions, which are the characteristic feature of tropical regions. The crop requires relatively less water than other important cereals such as maize and wheat. However, yield potential of the crop is significantly limited due to drought and heat stresses within the tropics and subtropics necessitating sorghum breeding for drought tolerance and productivity (Blum, 2005).

Drought is one of the most important factors that affect crop production worldwide. Climate changes will increase the frequency of drought and flood, particularly in many countries in Africa. There is indication that climate change may lead to a change in the frequency and severity of drought events. For instance, by 2050, water shortages are expected to affect 67% of the world's population (Ceccarelli et al., 2004). Drought can occur at any stages of the crop development. However, in the arid and semi-arid tropics, the probability of drought is highest at the start and end of the growing season. Drought stress at the beginning of the growing season will severely affect plant establishment. If drought occurs at flowering, or in the grain filling stages, it may result in reduced yield, or complete crop failure (Tumwesigye and Musiitwa, 2002).

Drought contributes to poor crop performance and yield. Countries in arid and semi-arid tropics usually experience insufficient, unevenly distributed, and unpredictable rainfall. At one point rain may be abundant and perhaps wasted through runoff; in some years much rain may fall completely outside the growing season. In other years, in adequate mid-season rain may fall after crops have germinated, causing crop failure. Although drought stress at the beginning of the growing season may severely affect plant establishment, plants tend to recover soon when late rain fall levels are adequate (Ramu et al., 2008). Consequently, crops are prone to periodic moisture stress in one way or another because of the aforementioned realities (Twomlow et al., 2008). The impact of moisture stress on crop yield is dependent on the stage of plant development (Tuinstra et al., 1997; Kebede et al., 2001). Anthesis and grain filling stages appear to be the most vulnerable growth stages; occurrence of drought at these stages may result in reduced yield and/or complete crop failure (Younesi and Moradi, 2009).

Crop production is constrained by several biotic, abiotic and socio-economic factors. Amongst the most important abiotic constraints, drought is the most important. Therefore, understanding of the physiological mechanisms

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and genetic control of drought in crops is important as a base for improving the production and productivity of crops in the arid and semi-arid tropics. In this article, physiological mechanisms, genetic control and breeding methods of drought tolerance in crops have been reviewed.

EFFECTS OF DROUGHT ON GROWTH AND DEVELOPMENT OF CROPS

In the semi-arid tropics where dryland farming is practiced, drought is a common phenomenon that occurs at different periods during the growing season (Blum, 1988). There is also a high season-to-season variability of rainfall, temperature, and radiation in the tropics. Agricultural conditions greatly vary in topography, soil, existing agricultural practices, and other associated biotic stress factors (Chapman et al., 2000b).

Drought is a combination of stress effects caused by high temperatures (Prasad et al., 2008) and a lack of water (Campos et al., 2004). Evapo-transpiration is the major driving force that affects the soil, plant, and atmospheric continuum of the hydrologic cycle. In earlier studies, predictions of drought were mainly based on the amount and distribution of precipitation (Blum, 2011). However, in recent studies soil moisture balance and soil characteristics have been introduced in the assessment of drought. Lack of adequate soil moisture, or water deficit, affects the ability of plants to grow and complete a normal life cycle (Moussa and Abdel-Aziz, 2008). Drought can have major consequences on growth, development and yield of crops by affecting several physiological, morphological and biochemical processes (Simpson, 1981). It is the major cause of poor crop performance and low yields, and sometimes it causes total crop failure. In the tropics, the probability of drought is highest at the start and the end of the growing season.

Drought can occur at both seedling, pre-flowering and post-flowering stages of development, and has the most adverse effect on yield (Tuinstra et al., 1997; Kebede et al., 2001). Drought stress at the seedling stage of development will severely affect plant establishment (Baalbaki et al., 1999). If it occurs at flowering, or in the grain filling stages, it may cause reduced yields, or complete crop failure (Blum, 1996). Researchers have classified drought as either pre- or post-flowering stress. The reactions of genotypes to these stresses are variable and controlled by different genetic mechanisms. Pre-anthesis moisture stress has effects on yield components such as stand count, tillering capacity, number of heads and number of seeds per head, while post-anthesis moisture stress affects transpiration efficiency, CO_2 fixation and carbohydrate translocation. The latter factors, in turn, results in low yields and premature plant senescence (Thomas and Howarth, 2000; Xin et al.,

2008).

PHYSIOLOGICAL MECHANISMS OF DROUGHT TOLERANCE

Levitt (1980) defined drought resistance as mechanisms of drought avoidance, recovery, survival and tolerance. These drought tolerance mechanisms are associated with plant survival and production. Drought avoidance is defined as the ability of plants to conserve water at the whole plant level through decreasing water loss from the shoots or by more efficiently extracting water from the soil (Ludlow and Muchow, 1990). However, drought tolerance is defined as the ability of plants to withstand water deficit while maintaining appropriate physiological activities to stabilize and protect cellular and metabolic integrity at tissue and cellular level (Tuinstra et al., 1997; Xiong et al., 2006).

Survival is the ability of the crop to survive drought, irrespective of the yield it produces, while production is the ability of the crop to grow and yield under water stress conditions (Levitt, 1980). Drought tolerance is a complex quantitative trait influenced by many genetic and environmental factors (Ceccarelli et al., 2004). The responses of different plants, species, or genotypes to drought are variable in relation to developmental stage, duration of drought, and evolutionary adaptation of the crop (Sanchez et al., 2002). In sorghum, for example, varieties that are adapted to arid and semi-arid environments showed higher drought tolerance than varieties of humid origin (Blum and Sullivan, 1986). Several studies have been conducted in understanding the mechanism of drought resistance in crops and in identifying essential traits for drought tolerance (Blum, 2011). Drought resistance, therefore, involves the interaction of different morphological structures, physiological functions, and biochemical expressions (Mitra, 2001; Borrell et al., 2006).

LEAF ROLLING AND STOMATAL CONDUCTANCE

In plants, stomatal conductance and leaf rolling have been found to be reliable physiological indicators of drought tolerance (Kadioglu and Terzi, 2007). Stomatal conductance and leaf rolling are strongly associated with leaf water potential (Bittman and Simpson, 1989). On the other hand, these two mechanisms are controlled by different factors because stomatal conductance is controlled by soil moisture dependent root signals, while leaf rolling is controlled by leaf water potential (Dingkuhan et al., 1999). The strong correlation of leaf rolling and leaf water potential allows breeders to use leaf rolling as a visual scoring criterion for selecting for drought resistance in plants (Hsiao et al. 1984). The rolling of leaves usually occurs following the reduction in

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leaf water potential. However, the degree of leaf rolling depends on the ability of the plant to adjust osmotically at low leaf water potential (Flower et al., 1990). Plants with high osmotic adjustment develop less leaf rolling, and hence, reduced leaf rolling is considered as an indicator of a greater degree of desiccation avoidance, through a deep root system (Hsiao et al., 1984).

Drought tolerant genotypes exhibit lower stomatal conductance associated with increased leaf temperature, which gives rise to high transpiration efficiency and lower carbon isotope discrimination (Khan et al., 2007). The increased leaf temperature and transpiration rate are due to a controlled transpirational cooling system induced by stomatal closure. The drought susceptible genotypes, on the other hand, show higher stomatal conductance and lower leaf temperature results in lower transpiration rates (Khan et al., 2007). The yield difference in stress and stress-free genotypes may be due to the negative relationship between yield potential and drought resistance in cereals. It is most likely explained by reduced assimilate translocation, due to drought resistance mechanisms, such as stomatal closure and reduced leaf area.

However, there is a lack of consensus on the benefits of the two traits of leaf rolling and stomatal conductance as drought resistance mechanisms. Leaf rolling has a detrimental effect on transpiration rate through changes in leaf stomatal conductance, and reduction in effective leaf area (Redmann, 1985). In addition, leaf rolling enhances stomatal closure by increasing leaf resistance to water loss. However, Heckathorn and DeLucia (1991) argued that leaf rolling had positive effects on reducing leaf temperature and loss of water by decreasing the incident irradiation. Stomatal closure alone causes a 70-80% decrease in transpiration rate in crops; however, leaf rolling causes a decrease of only 2% of normal transpiration rate (Heckathorn and DeLucia, 1991). Therefore, leaf rolling has less value in reducing water loss than stomatal closure. However, leaf rolling may increase the survival of plants by enhancing stomatal closure under extreme drought conditions (Heckathorn and DeLucia, 1991). The significance of using these traits as physiological indicators of plant drought adaptive mechanisms depends on the crop species and the environment. Under conditions where there are no sophisticated instruments to measure transpiration efficiency and stomatal conductance, leaf rolling is good indicator of drought tolerance.

ROOT CHARACTERISTICS

Roots are the primary plant organ affected by drought stress and other environmental stresses of the soil (Prince et al., 2002). Sorghum crown roots grow about 2 to 3 cm per day (Routley et al., 2003) and root growth is mainly affected by the amount of carbon partitioned to the

roots, although it varies with environmental and genetic factors (Blum, 2004). Sorghum roots may grow to depths of 1 to 2 m by the booting stage, and can efficiently extract water to a lateral distance of 1.6 m from the plant (Routley et al., 2003). Root growth in sorghum terminates at flowering stage; however, it is more prominent in a senescent than in nonsenescent sorghum genotypes (Robertson et al., 1993). Bawazir and Idle (1989) reported variation in root anatomy and morphology, among sorghum genotypes. Genotypes that have large number of seminal roots, large vessel diameter in both seminal and nodal roots showed better survival rate under drought stress conditions. Similarly, Habyarimana et al. (2004) found that the drought tolerance traits displayed by the genotypes were related to drought avoidance mechanisms. These, in turn, are associated with deep root system, which enables plants to exploit moisture from the deeper soil horizons.

Ekanayake et al. (1985) indicated that drought stress tolerance was found to be highly associated with root characteristics such as root thickness, root length density, number of thick roots, root volume, and root dry weight. It was also found that number of thick root, root thickness, and root length density were highly associated with leaf water potential and field visual drought scoring using drying leaf. Drought stress adapted plants are often characterized by deep and vigorous root systems (Blum, 1997). Nour et al. (1978) also reported root weight is the best and easiest attribute to determine drought tolerance in grain sorghum. Matsuura et al. (1996), on the other hand, reported a positive correlation between drought tolerance and root length in sorghum and millet (*Pennisetum glaucum*). Moreover, Plaut et al. (1996) and Pace et al. (1999) reported that seedlings under water stress caused an increase in root length with reduced diameter. Root depth, root length density, root distribution were reported as drought tolerance contributing traits (Taiz and Zeiger, 2006).

Drought is often associated with nutrient availability and the capacity of roots to absorb the available nutrients. Ludlow and Muchow (1990) indicated that greater root activity under intermittent drought should enhance crop stability by reducing the incidence of water deficits. Egilla et al. (2001) and HongBo et al. (2006) reported the significance of potassium (K) in improving drought resistance and root longevity. Shao et al. (2005) also reported the importance of mineral elements, such as K^+ and Na^+ for root signal transduction function. Shangguan et al. (2005) further denoted that the hydraulic conductivity of roots can be mainly affected by nitrogen and phosphorous nutrients. Hydraulic conductance in sorghum is primarily dependent on the number of fully functional nodal roots (Blum et al., 1977). In moisture stress conditions, plants with sufficient P supply exhibited higher hydraulic conductivity than P deficient plants. Therefore, plants with sufficient P are found to be more droughts tolerant, and also have a higher ability to

recover after drought.

OSMOTIC ADJUSTMENT, DEHYDRATION TOLERANCE AND TRANSPIRATION EFFICIENCY

Osmotic adjustment is another major physiological drought adaptive mechanism in plants (Izanloo et al., 2008). Sorghum and millet landraces, which are collected either dry or humid environments show variation in osmotic adjustment (Blum and Sullivan, 1986). Landraces that come from drier regions show greater osmotic adjustment than landraces from humid regions. The assumption is that through the course of evolution the drier environments provided sufficient selection pressure for osmotic adjustment. Landraces with higher osmotic adjustment are characterized by their dwarf nature with high rates of transpiration and low rates of leaf senescence under stress (Blum and Sullivan, 1986).

Osmotic adjustment improves crop productivity through delaying leaf rolling and leaf tissue death (Blum, 1996). As leaf rolling and leaf senescence decreases, the effective leaf area for photosynthesis increases. In a study on sorghum genotypes, those with high osmotic adjustment exhibited a 24% higher yield than genotypes with low adjustment, when exposed to a post-anthesis drought stress (Ludlow et al., 1990). The yield difference observed was both in grain size and grain number, and it was associated with higher harvest index. Similarly, a 20% dry matter yield increase has been observed in legume species that maintained turgor through osmotic adjustment (Amede and Schubert, 2003). The contribution of osmotic adjustment to reducing yield losses varies with the intensity and duration of the stress (Hsiao et al., 1984). In general, yield reduction of stressed plants compared with non-stressed plants is due to the plant's additional energy requirements for osmotic adjustment (Mitra, 2001; Blum, 2005).

Blum (1988) defined dehydration tolerance as the capacity of the plant to maintain higher turgor potential in the plant cell under moisture stress conditions. Dehydration tolerance is usually measured by tissue's water level, which is expressed in terms of water potential (Blum, 2011). This characteristic is highly associated with cell membrane stability to maintain high level of cell water potential under drought condition (Singh et al., 2008). Crops that exhibit high dehydration tolerance have the capacity to maintain physiological and biochemical functions; regardless of the magnitude of the water stress. In other words, physiological and biochemical activities of the cell, which are essential for growth and yield of the plant are not strictly constrained by water stress (Huang et al., 1997). However, dehydration sensitive plants accumulate solutes and macromolecules in the intracellular membrane, reduced loss of water through dehydration and alteration in the cellular membrane (Chaves and Oliveira, 2004). This, in turn, has

an influence in the performance of the plants in terms of growth and yield.

Transpiration efficiency (TE) is defined as biomass accumulation per unit water transpired (Xin et al., 2008). Variation in TE within species has been demonstrated for several C_3 plant species such as wheat, barley, rice, cotton, beans, tomato, and sunflower (Merah et al., 2001; Rebetzke et al., 2002; Teulat et al., 2002; Lambrides et al., 2004; Impa et al., 2005; Stiller et al., 2005). Genetic variation in TE has also been found in sorghum using gas-exchange properties, traditional lysimetric assays, and field evaluation (Hammer et al., 1997; Mortlock and Hammer, 1999). Among sorghum genotypes significant variation in TE and water treatments has been observed (Mortlock and Hammer, 1999). Sorghum genotypes with low internal CO_2 concentration and enhanced photosynthetic capacity may be associated with high TE (Xin et al., 2008). High TE was strongly correlated with increased biomass accumulation, rather than with reduced water use (Xin et al., 2008).

Solute accumulation and storage sugar

Solutes are low-molecular-weight and highly soluble compounds that are usually nontoxic even at high cytosolic concentrations. Generally they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of reactive oxygen species, stabilization of membranes, and native structures of enzymes and proteins (Farooq et al., 2009). In sorghum, proline (Sivaramakrishnan et al., 2006), glycinebetaine (GB) (Yang et al., 2003) and sugars functions as osmolytes that protect cells from dehydration (Wood and Goldsbrough, 1997). GB accumulation in cells can assist plants to either maintain water within cells or protect cellular component from dehydration (Yang et al., 2003). However, the genetic and metabolic basis of variation in GB accumulation is not well understood in sorghum (Borrell et al., 2006). Grote et al. (1994) reported that a recessive allele of a single locus is associated with non-accumulation of GB in sorghum genotypes.

Accumulation of free proline in water-stressed sorghum leaves is related to the ability of a cultivar to recover from stress, possibly due to proline's role as a source of respiratory energy in the recovering plant (Blum and Ebercon, 1976). In wheat, accumulation of proline (Shao et al., 2006a) and anti-oxidative enzymes (Shao et al., 2005) has been reported in both wild and cultivated species. Different wheat genotypes have different visible water threshold levels resulting in diverse responses to drought in terms of proline and anti-oxidative enzyme accumulation (Shao et al., 2006b). Proline comprises 18% of the osmotic pool in chickpea (Amede and Schubert, 2003). A strong accumulation of proline increases the cell solute concentration, resulting in

increased water potential in the tissue through osmotic adjustment. Alternatively, the expression of anti-oxidative enzymes serves as a signal transduction for gene expression, and hence, proteins are synthesized, which control metabolism effluxes (Shao et al., 2005). Evaluation of rice genotypes under *in vitro* drought induced conditions revealed a significant accumulation of proline and total soluble sugars in the leaves (Vajabhaya et al., 2001). The tolerant lines showed a continuous increase in proline level for five weeks after the stress was induced and started to decline after six weeks under drought. The solute concentrations decreased to normal levels when plants were allowed to recover from drought stress (Vajabhaya et al., 2001).

Grain formation and development in crop plants is dependent on assimilates produced by photosynthesis after anthesis or assimilates stored mainly in the stem before anthesis. Wheat genotypes revealed genotypic variation in the relative importance of pre-anthesis assimilates and post-anthesis photosynthesis to drought resistance (Inoue et al., 2004). A relatively high photosynthetic rate during grain filling under water stress was observed in drought resistant cultivars relative to susceptible cultivars. Moreover, the drought susceptible cultivars were much more reliant on remobilization of pre-anthesis assimilates stored in the stem to fill the grain as opposed to the resistant cultivar (Inoue et al., 2004). This demonstrates that, under moisture stress, the pre-anthesis assimilates stored in the stem in the drought resistance cultivars are used to maintain a higher photosynthetic rate during the grain filling period.

Remobilization of pre-anthesis assimilates from the leaf and stem is one of the drought escape mechanism. In conditions where photosynthesis is inhibited by stress such as drought, heat, leaf diseases or shading, the demand for nutrient storage usually exists (Blum et al., 1997). A large yield sink produces a physiological load on the leaves and stem, and the impact of this load is intensified under drought stress when the demand for carbon from stored reserves increases (Khanna-Chopra and Sinha, 1988). However, Blum et al. (1994) indicated that there are cases where the utilization of stem reserves for grain filling is not dictated by the environmental conditions. Genetically, male sterile plants showed a twofold increase in assimilate storage in the stems, indicating that removal of a grain sink increases stem sugar (Fortmeier and Schubert, 1995). Leaf defoliation during anthesis promoted lodging, suggesting that it may be due to depletion of carbohydrates from the stem (Rajewski and Francis, 1991). Conversely, each plant sink events (plant height, flowering time and tillering) may increase sugar production potential in non-stress environments (Murray et al., 2008).

When demand for storage carbohydrates develops during grain filling, carbohydrate degrading enzymes are activated which degrade the large carbohydrate molecules into simple sugars which are in turn transported

to the cab to fill the grain (Blum, 1997). Consequently, higher non-reducing sugar contents have been observed in the stem juice of high biomass genotypes of sweet sorghum at maturity as compared to the low yielding genotypes (Channappagoudar et al., 2007). This low level of non-reducing sugar in low yielding genotypes has been attributed to a decrease in invertase enzyme activity. This finding corroborates with the suggestion that reduced level of sucrose-degrading enzymes appears to be a requirement for the accumulation of sucrose in the stems of sorghum (Tarpley et al., 1994). However, this decline cannot account for the difference in stem sucrose content between sweet stem and grain sorghum. High sugar accumulation, mainly sucrose, was observed in the stem of non-senescence sorghum genotypes (stay-green) at the flowering stage (Kouressy et al., 2008a). This may delay the onset of senescence and maintain a high photosynthetic rate during the grain filling stage, and hence, reducing competition for assimilates between the grain and vegetative organs. Under conditions of severe terminal drought, this stem sugar reserve may be translocated to the developing grain.

STAY-GREEN / NON-SENESCENCE

Leaf senescence is a programmed cell death resulting from drought and other environmental stress factors. It is characterized by loss of chlorophyll and progressive decline in photosynthetic capacity (Tuinstra et al., 1997; Crasta et al., 1999; Tao et al., 2000). Premature plant tissue death usually occurs when plants are subjected to water stress during the grain filling period in sorghum (Rosenow and Clark, 1981). Stay-green, on the other hand, is a post-anthesis drought resistance trait in plants that provides resistance to pre-mature leaf senescence to the plant under severe moisture stress condition during grain filling stage. It contributes to an improved yield and yield stability under moisture stress condition (Tao et al., 2000). However, it reduces the source-sink translocation from leaves to grain.

Stay-green is associated with a higher level of chlorophyll content, cytokinin, and leaf nitrogen concentration under moisture stress conditions. The stay-green lines show higher levels of chlorophyll content than the normal lines (Xu et al., 2000). The visual scoring of leaf and plant senescence for the stay-green response as proposed by Wanous et al. (1991) was validated by Xu et al. (2000). Say-green sorghum lines exhibited high levels of cytokinin, suggesting that the reduced senescence rate of the stay-green lines is in part due to a higher level of cytokinin (Thomas and Howarth, 2000). Furthermore, stay-green genotypes are also associated with higher leaf nitrogen concentration, particularly at flowering (Borrell and Hammer, 2000; Borrell et al., 2000a), and basal stem sugars (Duncan, 1984), than senescent genotypes. This

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suggests that the stay-green trait may possibly contribute to higher transpiration efficiency of non-senescent genotypes. However, leaf senescence is characterized by a loss of chlorophyll and progressive decline in photosynthetic capacity (Tuinstra et al., 1997; Crasta et al., 1999; Tao et al., 2000).

Greater green-leaf area duration has been observed to occur in stay green varieties during grain filling stage, and therefore, van Oosterom et al. (1996) described that stay-green trait as post-flowering green leaf area duration (GLAD). The stay-green sorghum lines appear to be the combined effect of three distinct factors namely, green leaf area at flowering, time of onset of senescence, and subsequent rate of senescence (Borrell et al., 2000a). Large variations have been reported in the proportions of green-leaf area among different genotypes as a result of combined effects of differences in onset and rate of senescence (Borrell et al., 2000a; Mahalakshmi and Bidinger, 2002). An increase in biomass of about 47% over and above that obtained from senescent genotypes has been reported in genotypes that express the stay-green trait under post-anthesis moisture deficit (Borrell et al., 2000b). A lack of grain yield differences observed by these authors among genotypes grown under irrigated conditions, suggests that the stay-green trait does not reduce yield under zero moisture deficit conditions.

Stay-green improves resistance to diseases and lodging (Tenkouano et al., 1993). In sorghum, genotypes with the stay-green trait continue to fill their grain generally under moisture stress conditions (Rosenow and Clark, 1981), exhibit improved resistance to charcoal rot (*Macrophomina phaseolina*) and induced lodging (Woodfin et al., 1988). However, Tenkouano et al. (1993) reported that the non-senescence and charcoal rot reaction are genetically independent.

GENETICS OF DROUGHT TOLERANCE

The purpose of studying the genetics of drought resistance in plants is to identify genetic factors that determine the productivity of crops under drought stress conditions. Advances in crop improvement under water-limited conditions are only possible if drought resistance traits are identified and selected for in addition to yield (Borrell et al., 2000a; Sanchez et al., 2002). Quantitative trait loci (QTLs) have been mapped on the 10 linkage groups of sorghum. They are involved in controlling traits related to yield and yield components, root systems, stay-green, plant height, flowering and maturity (Sanchez et al., 2002).

A number of traits related to drought resistance have been identified and mapped; however, the stay-green trait is recognized as the most crucial drought resistance trait in sorghum. Tuinstra et al. (1997) identified 13 genomic regions associated with post-anthesis drought tolerance in sorghum. Four QTLs were identified for yield

and yield stability, seven for duration of grain development and seed weight, and two for the stay-green trait. There are three stay-green gene sources (B 35, SC 56 and E 36-1) from which QTLs that have been mapped onto 10 linkage groups on sorghum (Kebede et al., 2001; Haussmann et al., 2002).

Tao et al. (2000) identified two stay-green QTLs located on linkage group B and I. Likewise, Crasta et al. (1999) and Xu et al. (2000) identified four stay-green QTLs and mapped two of the QTLs (*Stg1* and *Stg2*) on linkage group A, and the other two, *Stg3* and *Stg4* onto linkage group D and J, respectively. The stay-green QTLs were ranked based on their contribution to the stay-green phenotype as *Stg2*, *Stg1*, *Stg3*, and *Stg4* in their order of merit. Furthermore, Xu et al. (2000) also mapped three QTLs (*Chl1*, *Chl2* and *Chl3*) for chlorophyll content, and the map position coincides with the stay-green QTLs. The phenotypic association of the stay-green trait and chlorophyll content may be explained by the map position of these QTLs on the genome.

Many secondary factors, such as differences in flowering time, reproductive sink strength together with variation in the environmental factors alter the expression of the stay-green trait (Harris et al., 2007; Tao et al., 2000). Six maturity genes (*Ma$_1$*- *Ma$_6$*) have been identified, and mapped onto the sorghum genome. The dominant forms of these genes cause extreme lateness (Morgan et al., 2002). Two maturity QTLs are positioned near a stay-green QTL linkage group and the major independent maturity QTLs were found to be highly correlated with stay-green rating (Subudhi et al., 2000; Xu et al., 2000). Tropical genotypes are found to be dominant for all four loci (*Ma$_1$-Ma$_4$*) that control the time of flowering (Quinby, 1974). However, substituting the dominant maturity gene, *Ma$_1$*, to recessive *ma$_1$* converts a tropical sorghum to a temperate one that will flower in high latitudes (Major et al., 1990). Tuinstra et al. (1998) identified two QTLs that conditioned the expression of the stay-green trait. The physiological association of the maturity and stay-green trait is not well understood. The indistinct association between the two traits suggests that the earliness trait may work against reproductive sink strength during post-anthesis drought stress.

Walulu et al. (1994) found that the stay-green trait in sorghum is controlled by a major gene that expresses different levels of dominant gene action, depending on the environment. However, van Oosterom et al. (1996) studied the stay-green trait as a function of green leaf area duration (GLAD), which is affected by green leaf area at flowering, time of onset of senescence, and subsequent rate of senescence. It has been reported that the three stay-green components appeared to be inherited independently. The inheritance of the onset of leaf senescence was additive, and the senescence rate was dominant. Consequently, GLAD was found to be partially dominant. The expression of these three factors is also affected by many environmental factors, and

hence, the combined genetic effects of the three factors and the environmental factors should be considered when designing breeding programs for drought resistance (Borrell et al., 2000b; Mahalakshmi and Bidinger, 2002). Delayed senescence in sorghum is a valuable trait that improves genotypes adaptation to drought stress, grain filling and grain yield under stress.

Several genes are involved in drought stress tolerance in various plant species. The function of these genes is either protecting the cell from water deficit by the production of important metabolic proteins, or regulation of genes for signal transduction. The expression of the dehydrin gene, *dhn1* in sorghum as a response to water deficit was reported by Wood and Goldsbrough (1997). Expression of the *dhn1* gene in seedlings and pre-flowering sorghum was identical among genotypes, but genotypes showed variation in time of expression of the gene. This suggested that the expression of dehydrins is an important drought adaptation mechanism in sorghum.

The expression of genes related to water deficit in plants is found to be induced by water stress, desiccation, and abscisic acid (ABA). Yamaguchi-Shinozaki et al. (2002) observed a wide variation in the timing of induction and expression of drought related genes classifying the genes into two groups. The first group is responsible for proteins that function directly under stress tolerance, and the second group produces protein factors involved in the regulation of signal transduction and gene expression under drought (Yamaguchi-Shinozaki et al., 2002). Most of these drought-inducible genes are induced by ABA. However, various researchers have reported the existence of ABA-dependent, and ABA-independent, signal transduction cascades between the initial signal of drought stress and the expression of the genes (Shinozaki and Yamaguchi, 1997, 2000). Inhibition of lateral root development under moisture stress condition is reported as one mechanism of drought tolerance in plants (Xiong et al., 2006). The drought-induced inhibition of lateral root growth is partly mediated by abscisic acid. Plants that are sensitive to abscisic acid in lateral root growth are more drought tolerant than those insensitive to abscisic acid (Xiong et al., 2006). It was also found that abscisic acid insensitive plants have higher transpiration rates and lose water much faster than abscisic acid sensitive plants (Yamaguchi-Shinozaki et al., 2002).

Four QTLs for nodal root angle (*qRA1_5*, *qRA2_5*, *qRA1_8*, *qRA1_10*), three QTLs for root dry weight (*qRDW1_2*, *qRDW1_5*, *qRDW1_8*) and eight QTLs for root volume, root fresh weight and root dry weightwere identified by (Mace et al. (2012) and Rajkuma et al. (2013). Additionally, one of the root angle QTL are co-located with QTL for stay-green in sorghum and associated with grain yield (Mace et al., 2012). Recently two QTLs (*qRT6* and *qRT7*) associated with brace roots have been mapped on sorghum Chromosome 6 and 7. Brace roots significantly contribute to effective anchorage

and water and nutrient uptake during late growth and development and have a substantial influence on grain yield under water limited conditions (Li et al., 2014). The inheritance of root characters was controlled equally by both additive and dominant genetic effects (Ekanayake et al., 1985).

Leaf rolling is known as a typical response to water deficit in numerous species such as rice, maize, wheat and sorghum. The rolling of leaves is mainly controlled by major genes and several genes (*RL1* to *RL10*) were identified rice. *RL7*, *RL8* and *RL9* were among those which were assigned to their corresponding chromosomes with molecular markers. Single-recessive alleles of each gene exhibited rolled leaves (Zhang et al., 2009). Luo et al. (2007) identified a dynamically rolled leaf mutant (*RL10*) from a spontaneous mutation in rice. In maize, *RDL1*, a semi-dominant maize mutant gene controlled the upward curling of the leaf blade (Juarez et al., 2004). Zhang et al. (2009) isolated and characterized *SLL1* gene, a key gene controlling rice leaf rolling. *sll1* mutant plants have extremely incurved leaves due to the defective development of sclerenchymatous cells on the abaxial side.

BREEDING FOR DROUGHT TOLERANCE

Generating and selecting for new combinations of genes to produce genotypes with superior trait performances than those of existing genotypes, within the target environment, is the major objective of plant breeding (Chapman et al., 2003). In any breeding programme, defining the critical traits to improve grain yield in a given target environment is critical (Fernandez, 1992). Identification of important traits depends on the degree of influence of a trait on yield, expression of the trait at a whole plant level, the nature of the target environment (rainfall amount, distribution, onset and cessation, available soil water, nutrient status of the soil, and diseases), and economic environment (the requirements of grain quality and quantity). In maize, for example, it has been found that early flowering, crop water use efficiency and early vigour are important traits to breed for improve yield under drought condition (Richards, 1996).

The greater flexibility of sorghum in adapting to diverse climatic conditions has resulted in the evolution of tropical and temperate sorghum varieties. The tropical varieties are characterized by being tall, late maturing with low harvest indices, photoperiod sensitivity and poor population performance. They are generally adapted to low population levels and exhibit little response to improved agricultural practices (fertilization and mechanized harvesting). The temperate sorghum varieties, on the other hand, are characterized by dwarf stems, early maturity, high yields, and less dry matter per plant (Rao et al., 2002). In the early sorghum improvement

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programme, conversions of tropical varieties to temperate varieties were made by substituting two dominant alleles for height and three for maturity for their recessive counterparts. The conversion programme started with hybridization of tropical and temperate varieties followed by successive backcrossing (Acquaah, 2007).

In breeding for drought tolerance, a pure line selection method has been used in many national and regional sorghum improvement research programmes in Africa and Asia (Acquaah, 2007). However, pedigree and bulk selection methods are commonly used in most international and national breeding institutions. Pedigree selection in segregating populations derived from planned crosses is the dominant breeding strategy to develop pure line varieties and hybrid parents in sorghum (Dar et al., 2006). If the transfer of only a few traits relating to drought resistance to a high yielding cultivar is required, then backcrossing is the appropriate breeding method (Mitra, 2001).

Exploitation of heterosis by the production of hybrids is routine in most sorghum breeding programmes after the discovery of stable and heritable cytoplasm-nuclear male sterility systems in the crop. This discovery further enables large-scale production of commercial hybrid seed to be commercially viable (Dar et al., 2006). One study of the expression of hybrid vigour in grain sorghum revealed that there was an 84% increase in number of seed per plant, an 82% increase in grain weight, and a 12% increase stover weight in the hybrids relative to the better parent (Doggett, 1988).

Plant breeders have two basic approaches for breeding for drought resistance, direct and indirect breeding. Direct selection for drought is conducted under conditions where stress factors occur uniformly and predictably whereas indirect selection involves selection of genotypes under managed stress environments. However, environmental factors such as temperature and moisture are highly variable from one location to another and hence difficult to predict. Moreover, variation for stress tolerance actually exhibits a large environmental component or large genotype-by-environment interaction making direct selection for a physiological trait in a single environment difficult. As a result, indirect selection breeding is used as a preferred method where selection is made based on yield *per se*, or based on developmental traits or based on assessment of plant water status and plant function (Ludlow and Muchow, 1990).

In the past drought resistance screening was done under optimal conditions, because the maximum genetic potential of yield can only be realised under optimum conditions. Additionally, it was believed that a high positive correlation exists between performance under optimum and stress conditions (Tuinstra et al., 1997; Habyarimana et al. 2004). However, a high genotype by environment interaction may restrict the expression of the yield potential under drought condition (Chapman et al.,

2000a, b). Although, there is a yield penalty when selecting plants under drought condition in contrast to optimal environmental conditions, Richards (1996) and Tuinstra et al. (1997) suggested that selection under both optimal and drought conditions represents the ideal trial design to select for yield and yield stability, drought tolerance and expression of drought related traits. Hence, drought resistance and its impact on yield involve interaction between plant water relations and plant physiological functions. The interactions are further complicated by the frequency and duration of the drought, plant development stage and other stress factors such as low soil fertility and biotic stress factors.

CONCLUSION

Understanding the genetic and physiological basis of drought resistance in plants is essential when breeding for drought resistance. Lin et al. (1995) and Kouressy et al. (2008b) identified the importance of reduced plant size in terms of small and narrow leaf structures and genetic dwarfing of the plant for drought resistance. Reduction of leaf area index, through reducing the number of leaf and narrowing the leaf structure, also results in the reduction of the effective photosynthesis area. This in turn, reduce the amount of assimilates produced and the grain yield. However, it also reduces the amount of water lost through transpiration. Genetic dwarfing, on the other hand, increases the efficiency of plants in balancing the translocation of assimilates translocation between the developing grain and the vegetative organs (Kouressy et al., 2008b).

An early maturing genotype yields less compared to a late maturing genotype in a favourable environment. This is because drought escape by shortening the growing period is made at the expense of the crops genetic yield potential. Short cropping duration may not be an exclusive selection criterion *per se* because other factors may also be involved in affecting genotype performance under water stress (Blum et al., 1989). Stay-green is a valuable trait that improves genotype adaptation to drought stress, grain filling and grain yield under stress (Borrell et al., 2000b; Mahalakshmi and Bidinger, 2002), without a yield penalty under moisture deficit conditions as compared to osmotic adjustment and early maturity (Borrell et al., 2000a). The balance among these characters maintains adequate productivity by providing a spectrum of effective drought tolerance mechanisms.

Many researchers have proposed various characteristics related to drought resistance that could be used in breeding and selection programmes. However, comprehensive understandings of the physiological and genetic basis of adaptation in moisture stress conditions are still lacking. Moreover, the interaction between the different characteristics within a plant and the environment makes drought resistance breeding very complicated.

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Hence, the chances of breeding drought resistant cultivars with all the important characters are low. The choice of specific traits as selection criteria depend on the crop species, the heritability, and the ease of transfer of the traits. Moreover, since drought resistance is the interaction of different morphological, physiological, and biochemical traits, a combination of different resistance traits, rather than a single trait, should be used as the overall selection criterion.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Genetic diversity of traditional genotypes of barley (*Hordeum vulgare* L.) in Algeria by pheno-morphological and agronomic traits

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Barley ranks third after durum and bread wheat grain production in Algeria. In this study, genetic diversity of twenty nine traditional genotypes of barley was assessed using twenty seven pheno-morphological and agronomic traits in presence of four controls. Very high significant differences were found between genotypes for all quantitative characters statistically analyzed, indicating existence of a great variability within the germplasm. Some traditional genotypes differed from all controls by their better mean values of 1000 grain weight, tiller number per spike, grain number per spike and grain protein content. The long-cycle genotypes had higher values of 1000 grain weight, awn length and plant height than those with short cycle. For quantitative traits, the principle component analysis showed that three components could describe 68.27% of total variances and the cluster analysis divided all six-rowed barley studied into three cluster groups. The following traits: 1000 grain weight, awn length, days to heading and maturity, grain width, grain number per spike, plant height, length of first rachis segment and grain length were those contributing more to variability among the genotypes and also but less strongly the tiller spike number per plant and the spike length. The awn length, the 1000 grain weight, the grain width and the plant height were positively correlated with highly significant correlations between the majority of them. On the qualitative traits, the growth habit, the curvature of the first rachis segment and the lower leaf sheath hairiness were the traits which varied more between all genotypes.

Key words: Qualitative traits, quantitative traits, variability.

INTRODUCTION

Barley is among the most important cereal in the world. It is one of the most ancient crops among the cereals and has played a significant role in the development of agriculture (Ullrich, 2011).

In Algeria and at the beginning of the nineteenth century, barley came at the head of cultures by its importance; it was intended for human consumption and was used as fodder complement. At present, barley ranks third in

Algeria from the point of view growing area and production. It represents currently the main food of sheep.

The introduction during years 1965-1970 of seeds and plantations of varieties with high genetic potential caused the regression of some traditional cultivars. The new varieties of barley adopted in Algeria remain marginal because of their bigger sensibility at the climatic variations.

Jilal (2011) indicates that throughout time, landraces recognized as valuable sources of resistance to pest, diseases and abiotic stresses have been increasingly replaced by commercial cultivars. According to Lasa et al. (2001), specific adaptation traits exist in autochthonous barley and have not been totally incorporated into modern cultivars. Evaluating genetic diversity in cultivated plants for plant breeding programs and heritable resources protection has a vital usage (Khajavi et al., 2014). Determining the level of variation within and among barley populations is an essential step towards conserving genetic resources and developing future strategies for plant improvement (Backes et al., 2009).

Genetic diversity among and within plant species is in danger of being reduced (Eshghi and Akhundova, 2010). Some studies on genetic diversity of barley have focused on phenological and quantitative traits (Assefa and Labuschagne, 2004; Manjunatha et al., 2007; Shakhatrech et al., 2010; Mekonnon et al., 2015). Diversity in barley based on agronomic traits was measured by several authors (Eshghi and Akhundova, 2010; Muhe and Assefa, 2011; Amabile et al., 2013). According to Eshghi and Akhundova (2010), many authors showed that grain yield is an ultimate product of the action and interaction of number of components such as number of tillers, number of grains per spike, 1000-grain weight, plant height, harvest index and etc.

Few works exist on the local resources of cultivated barley in Algeria. On the species *Hordeum vulgare*, studies concerned generally the local approved varieties "Saïda" and "Tichedrett" and hence the diversity within the local materiel is not known.

This work was done with the objectives to conduct the morphological characterization and to estimate the extent and variability in pheno-morpho-agronomic traits on the barley genotypes of Southern Algeria.

MATERIALS AND METHODS

The germoplasm studied on barley consisted of twenty nine genotypes coming from various regions of Algeria. The collection sites were divided into western, central and eastern regions of Algerian Southern. 11 genotypes were recovered from ICARDA (Syria). They concerned the following regions: Biskra with five Genotypes (1, 7, 9, 10 and 16); El Bayadh (8 and 13); Ouargla (11 and 12) and Bechar (14 and 15). Other genotypes were collected by researchers of INRAA within the following regions: Adrar (2, 4, 20, 21, 22 and 24); Touggourt (17, 18, 19, 25, 26 and 27); Ghardaïa (5) and Tamanrasset (28, 29, 30, 31 and 32). All the genotypes

were six-rowed barley except the Genotype (14) from Bechar which was barley with 2 rows.

Tamanrasset is located in the Central Sahara and Adrar in South-Western of it. Touggourt, Biskra and Ouargla are regions of the Low Sahara which is a geographic region of north eastern Sahara of Algeria. Bechar is a region of southwest of Algeria. El Bayadh is located on high steppe plains of the southwest of Algeria. Ghardaïa is a part of M'zab which is located between the Low Sahara and the great western erg.

The study was conducted at the Institute of Algerian Agricultural Research of Baraki (Algiers) situated in the plain of Mitidja (with an average rainfall exceeding 500 mm). Planting occurred on 8 December in 2011. Genotypes were evaluated in a sampling random design on three plots in presence of four controls Barberousse or Genotype 3 (France), Pane or Genotype 6 (Spain) and two approved Algerian varieties Saïda (23) and Tichedrett (33). Lines were 4.80 m each, spaced out by 40 cm. With 25 seeds by line, the distance between plants was 20 cm.

Plant height (HPL) (cm), spike length (HEP) (cm), awn length (LBA) (cm), first rachis segment length (LPA) (mm), lower lemma length in sterile spikelet (LGI) (mm), tiller spike number per plant (NTE), grain number per spike (NGE), spikelet number per spike (NEE), days to heading (DEP), days to maturity (DC), 1000 grain weight (PMG) (g), grain length (LOG) (mm), grain width (LAG) (mm) and grain protein content (PRO) (%) were the quantitative traits evaluated.

The qualitative traits were: Growth habit, Lower leaf sheath hairiness, port of flag leaf, shape of spike, spike density, lemma awn barbs, pigmentation of awn tips, glume length compared to grain, lemma type in grain, shape of lemma type in sterile spikelet, curvature of first rachis segment, rachilla hair type and disposition of lodicules in the fertile grain. A random selection of 30 plants was considered to study these following characters: HPL, HEP, LBA, LPA, LGI, NTE, NGE, NEE, LOG and LAG (ten per plot). Also, to determine the degree of fluctuation in the qualitative characters within every genotype, 30 observations by genotype were taken for every character. The test was led without irrigation or fertilization. The texture of the soil was a sandy clay loam texture. The studied characters (quantitative and qualitative) were in their majority, those described by IBPGR (1994) and Niane et al. (1999).

The analysis of variance (ANOVA One-way) was performed by Fisher's least significant difference (LSD) method to test the significance difference between means. The data was statistically analyzed by the Gen Stat Discovery (Edition 3, Stat Soft Inc.) and concerned only characters with 30 repeated measurements (HPL, NTE, HEP, LBA, LPA, NEE, LGI, NGE, LAG, LOG) (Tables 1 and 2). Correlations (Table 3), principal Component Analysis (Figure 1 and Table 4), cluster analysis (Figure 2) were obtained by STATISTICA (Data analysis Software System, version 8, Stat Soft Inc.). Correlations were performed based on fourteen quantitative characters (HPL, NTE, HEP, LBA, LPA, NEE, LGI, NGE, LAG, LOG, PMG, DEP, DC and PRO). Principal component and cluster analyzes were taken on: HPL, NTE, HEP, LBA, LPA, NEE, NGE, LAG, LOG, PMG, DEP and DC.

The cluster analysis was adopted with the Ward's method as a clustering algorithm (Ward, 1963). The grain protein content was determined from the nitrogen content, tested by Kjeldahl method (AFNOR, 1985). It is expressed in percentage by weight referred to dry matter.

RESULTS

Very highly significant differences were found between genotypes for all quantitative traits statistically analyzed, thus indicating a strong genetic variability within the

Table 1. ANOVAs of agro-morphological traits in barley genotypes.

S.O.V.	DF	HPL	NTE	HEP	LBA	LPA	NEE	LGI	NGE	LAG	LOG
Genotype	32	26.45 ***	8.75***	41.7***	105.08***	46.79***	10.3***	3.75***	11.38***	89.23***	17.87***
Error	957	82.2	50.14	0.87	1.11	0.191	2.67	1.84	93.3	0.08	0.53
CV %		9.0	42.8	13.7	10.3	14	16.3	14.2	20.6	9.2	7.4
SE		9.07	7.08	0.94	1.06	0.44	1.63	1.36	9.66	0.28	0.73
SED		2.34	1.83	0.24	0.27	0.11	0.42	0.35	2.49	0.07	0.19
LSD		4.59	3.59	0.47	0.53	0.22	0.83	0.69	4.89	0.14	0.37

***: Significant at $P<0.001$; SE: Standard Error; SED: Standard Errors of means; LSD: Least significant differences of means at 5% level.

Table 2. Means and group means of agro-morphological traits in barley genotypes.

N°	HPL	NTE	HEP	LBA	LPA	NEE	LGI	NGE	LAG	LOG
1	87.33^L	22.73^{BC}	5.37^{LM}	11.17^G	2.84^{KLM}	$9.2IJKL$	$9.6^{ABCDEFG}$	$49.13^{DEFGHIJ}$	2.61^M	10.27^{CDE}
7	100.77^{GHI}	$15.2^{GHIJKLM}$	6.7^{GHI}	9.67^H	3.11^{GHI}	10.07^{DEFGH}	7.73^I	$46.03^{HIJKLMN}$	3.31^{HI}	10.0^{EFGH}
9	105.27^{DEFG}	$14.87^{GHIJKLM}$	7.42^{CDE}	12.41^{BC}	3.58^{CD}	9.3^{HIJKL}	10.13^{AB}	40.06^{OP}	3.67^{CD}	10.03^{DEFGH}
10	109.1^{ABCD}	25.3^B	7.57^{CD}	11.37^{FG}	2.42^{PQR}	10.86^{CD}	10.23^A	55.63^{AB}	3.38^{GH}	10.55^{BC}
16	101.77^{FGH}	15.4^{GHIJKL}	7.74^{BCD}	12.03^{BCDE}	3.78^C	9.73^{FGHI}	$9.63^{ABCDEFG}$	42.33^{LMNOP}	3.71^{BC}	9.59^{IJKLM}
2	93.58^{JK}	16.43^{FGHIJK}	6.21^{JK}	$7.73NO$	2.77^{LMN}	9.6^{GHIJ}	10.03^{ABC}	$50.53^{CDEFGHI}$	3.28^{HI}	10.06^{DEFG}
4	93.17^{JK}	13.16^{KLM}	5.33^{LM}	7.89^{MN}	2.81^{LM}	9.76^{FGHI}	$9.86^{ABCD}E$	48.93^{EFGHIJ}	2.44^{NO}	9.17^{NOP}
20	87.13^L	$16.1^{FGHIJKL}$	4.02^O	8.58^{JK}	2.29^R	12.13^A	$9.5^{BCDEFGH}$	58.93^A	3.22^{IJ}	8.95^P
21	90.93^{KL}	14.2^{JKLM}	5.34^{LM}	7.97^{LMN}	2.81^{LM}	10.2^{DEFG}	9.03^{GH}	52.56^{BCDE}	2.44^O	9.43^{LMN}
22	94.77^{JK}	$15.23^{GHIJKLM}$	6.15^{JK}	7.66^{NO}	3.17^{GH}	8.53^L	9.93^{ABCD}	43.5^{KLMNOP}	2.67^{LM}	10.06^{DEFG}
24	93.5^{JK}	13^{KLM}	5.28^M	8.09^{KLMN}	2.87^{JKLM}	10.03^{EFGH}	9.3^{DEFGH}	54^{BCD}	2.7^{LM}	9.35^{LMNO}
3	77.87^M	13.7^{JKLM}	7.04^{EFG}	9.5^{HI}	2.54^{OPQ}	10.66^{CDE}	9.3^{DEFGH}	$46.26^{HIJKLMN}$	2.25^P	9.04^{OP}
6	96.73^{IJ}	14.5^{IJKLM}	5.76^{KL}	11.63^{EFG}	2.32^{DEF}	10.16^{DEFG}	9.13^{FGH}	45.53^{JKLMN}	2.81^L	10.02^{EFGH}
23	107.73^{BCDE}	16.23^{FGHIJK}	7.07^{EFG}	12.55^B	3.63^{CD}	9.26^{HIJKL}	8.9^H	39.03^P	3.83^{AB}	9.8^{GHIJK}
33	87.17^L	14.7^{HIJKLM}	4.8^N	13.62^A	4.44^A	10.86^{CD}	9.23^{EFGH}	$48.2^{EFGHIJK}$	2.67^{LM}	9.67^{HIJKL}
5	106.13^{CDEF}	13.73^{JKLM}	7.55^{CD}	9.04^{IG}	3.44^{DEF}	10.43^{CDEF}	9.4^{CDEFGH}	$49.23^{DEFGHIJ}$	2.65^M	10.39^{BCD}
8	103.37^{EFGH}	11.8^M	7.07^{EFG}	11.98^{CDE}	3.47^{DE}	$8.86JKL$	9.4^{CDEFGH}	39.1^P	3.54^{DEF}	$10.7AB$
13	109.27^{ABCD}	20.13^{CDE}	7.48^{CDE}	11.95^{CDE}	3.14^{GHI}	9.13^{IJKL}	9.93^{ABCD}	41.63^{MNOP}	3.87^A	10.74^{AB}
11	109.4^{ABCD}	12.56^{LM}	7.07^{EFG}	11.83^{DEF}	4.08^B	$9.3^{HIJK}L$	$9.6^{ABCDEFG}$	$46.46^{GHIJKLM}$	3.39^{GH}	10.57^{BC}
12	100.57^{HI}	14.3^{JKLM}	7.3^{DEF}	11.69^{EFG}	3.71^C	$9IJKL$	9.23^{EFGH}	44.93^{JKLMNO}	2.58^{NO}	10.99^A
14	100.00^{HI}	29.3^A	10.4^A	8.32^{KLM}	2.79^{LM}	12.13^A	$9.46^{BCDEFGH}$	30.2^Q	3.16^{IJ}	9.46^{KLMN}
15	109.13^{ABCD}	$15.13^{GHIJKLM}$	7.77^{BCD}	12.24^{BCD}	3.74^C	10.03^{EFGH}	9.8^{ABCDEF}	41.53^{NOP}	3.71^{BC}	9.68^{HIJKL}
17	107.73^{BCDE}	$14,43^{JKLM}$	8.11^B	11.91^{CDE}	3.29^{EFG}	10.8^{CDE}	9.4^{CDEFGH}	$47.73^{EFGHIJK}$	3.46^{FG}	9.87^{FGHI}
18	112.63^A	14.3^{JKLM}	7.68^{BCD}	$12 .16^{BCDE}$	2.74^{LMNO}	10.8^{CDE}	9.4^{CDEFGH}	$46.86^{FGHIJKL}$	3.48^{FG}	9.5^{JKLMN}
19	110.27^{ABC}	13.63^{JKLM}	7.46^{CDE}	12.28^{BCD}	3.24^{FGH}	9.46^{GHIJ}	$9.6^{ABCDEFG}$	40.56^{OP}	3.52^{EFG}	9.83^{GHIJ}
25	106.4^{CDE}	17.03^{EFGHIJ}	7.48^{CDE}	11.98^{CDE}	2.35^{PQR}	11.16^{BC}	8.86^H	55.26^{ABC}	3.52^{EFG}	9.35^{LMNO}
26	100.53^{HI}	18.23^{DEFGH}	6.79^{GH}	7.88^{MN}	2.93^{IJKL}	10.76^{CDE}	$9.53^{BCDEFGH}$	50.7^{CDEFGH}	2.65^M	10.22^{CDEF}
27	101.7^{FGH}	21.46^{CD}	7.88^{BC}	8.39^{KLM}	3.1^{GHI}	11.7^{AB}	10.06^{ABC}	55.7^{AB}	2.26^P	9.2^{NOP}

Table 2. Contd.

28	111.83[AB]	14.1[JKLM]	7.07[EFG]	11.83[DEF]	4.08[B]	9.3[HIJKL]	9.6[ABCDEFG]	46.46[GHIJKLM]	3.63[CDE]	10.02[EFGH]
29	96.67[IJ]	18.33[DEFG]	6.55[HIJ]	7.65[NO]	2.55[NOP]	9.7[FGHI]	10.0[6ABC]	51.23[BCDEFG]	3.11[JK]	9.05[OP]
30	107.53[BCDE]	18.06[DEFGHI]	6.86[FGH]	7.33[O]	3.09[GHIJ]	10.73[CDE]	9.9[ABCDE]	51.6[BCDEF]	3.19[IJ]	9[OP]
31	100.8[GHI]	19.36[CDEF]	6.88[FGH]	9.21[HI]	3.05[HIJK]	8.76[KL]	9.86[ABCDE]	43.8[KLMNOP]	3.02[K]	9.26[MNOP]
32	107.27[BCDE]	19[DEF]	6.3[IJ]	8.46[KL]	2.66[MNO]	8.83[JKL]	9.33[DEFGH]	45.66[IJKLMN]	2.61[M]	9.25[MNOP]

Table 3. Correlation matrix on fourteen traits and 33 barley genotypes.

Variables	HPL	NTE	HEP	LBA	LPA	NEE	NGE	LGI	LAG	LOG	PMG	DC	DEP
NTE	0.04[NS]												
HEP	0.59***	0.38*											
LBA	0.38*	-0.22[NS]	0.21[NS]										
LPA	0.29[NS]	-0.36*	0.15[NS]	0.51**									
NEE	-0.16[NS]	0.33[NS]	0.15[NS]	-0.17[NS]	-0.34[NS]								
NGE	-0.26[NS]	-0.06[NS]	-0.55**	-0.34[NS]	-0.39*	0.38*							
LGI	0.10[NS]	0.28[NS]	0.11[NS]	-0.16[NS]	-0.01[NS]	-0.10[NS]	0.06[NS]						
LAG	0.65***	-0.02[NS]	0.39*	0.56**	0.27[NS]	-0.14[NS]	-0.37*	0.04[NS]					
LOG	0.22[NS]	-0.13[NS]	0.12[NS]	0.37*	0.28[NS]	-0.14[NS]	-0.13[NS]	-0.09[NS]	0.18[NS]				
PMG	0.49**	0.02[NS]	0.43*	0.76***	0.37*	-0.13[NS]	-0.58***	-0.00[NS]	0.73***	0.29[NS]			
DC	0.34[NS]	-0.07[NS]	0.43*	0.75***	0.39*	0.11[NS]	-0.41*	-0.25[NS]	0.43*	0.46*	0.60***		
DEP	0.26[NS]	-0.24[NS]	0.35*	0.70***	0.45**	0.09[NS]	-0.35*	-0.22[NS]	0.34[NS]	0.24[NS]	0.55**	0.84***	
PRO	0.12[NS]	-0.16[NS]	0.08[NS]	0.14[NS]	0.10[NS]	0.04[NS]	-0.09[NS]	-0.08[NS]	0.19[NS]	0.19[NS]	0.22[NS]	-0.02[NS]	-0.10[NS]

germplasm (Tables 1 and 2). The distinction between most of the genotypes was marked by at least one character (a separate group). The most distinct genotype is from Adrar (20) at which separate groups are noted on six characters (Table 2). The two-rowed barley (14) also distinguished by its highest values (group A) for characters: tillers spike number per plant (NTE), spike length (HEP), spikelet number per spike (NEE) and by the lowest average (group Q) in the grain number per spike (NGE). Seventeen genotypes gave tiller spike number per plant more than fifteen, exceeding "Pane", "Barberousse" and "Tichedrett (Table 2).

Barley with two rows produced more tillers than all six-rowed genotypes (29.3 tillers). Ten (10) genotypes gave a grain number per spike greater than 50, exceeding the four controls (Table 2). The highest value was registered at the genotype coming from Adrar (20) with 58.93 grains per spike. Seventeen genotypes gave a NGE greater than 40 and the three controls (Tichedrett, Pane and Barberousse). The lowest value of NGE was recorded to barley with two rows (30.2). Nine genotypes gave a 1000 grain weight (PMG) greater than 50 g and also witnesses "Pane" and

"Saïda". With "Tichedrett", five genotypes gave a PMG more than 40 g.

The better mean value of 1000 grain weight was registered at the Genotype 9 coming from Biskra with 60.9 g. The lowest value concerned 26 from Touggourt with 30 g. Twelve genotypes gave grain protein content exceeding 10% and thus better than controls "Pané" and "Barberousse (9.3 and 8.9% respectively). The highest value was 12.12% and concerned the Genotype 17 (from Touggourt), followed by Genotypes 2 and 21 (from Adrar) with 11.5 and 11.19%, respectively.

The correlation matrix (Table 3) revealed several
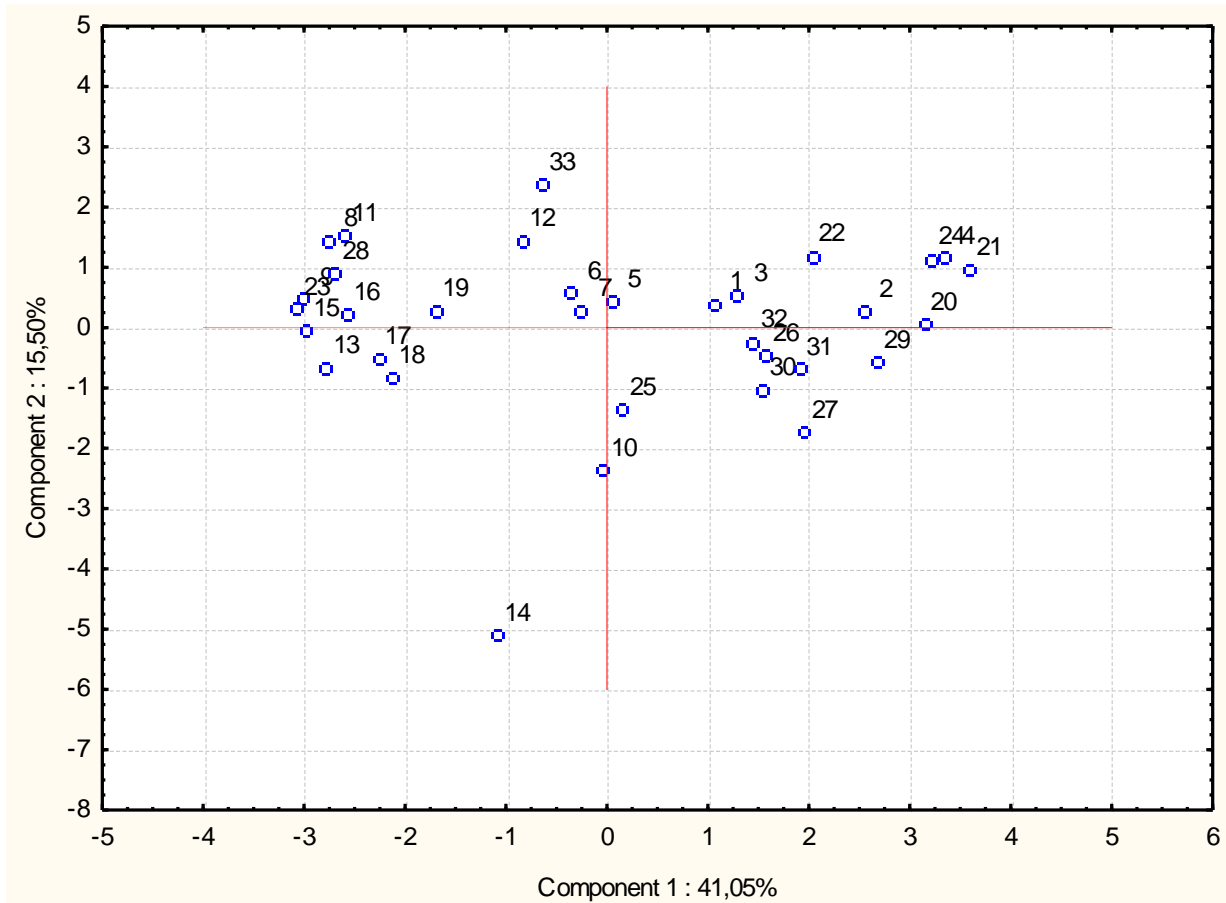
Figure 1. Distribution of genotypes based on the first two components.

Table 4. Principal component analysis (PC) of 33 barley genotypes based on twelve traits.

Parameter	PC 1	PC 2	PC 3
Eigen values	4.93	1.86	1.41
% of variance	41.05	15.5	11.73
Cumulative %	41.05	56.55	68.27
Characters	**Eigenvector**		
HPL	-0.630	-0.277	-0.338
NTE	0.123	-0.830	-0.154
HEP	-0.566	-0.654	-0.131
LBA	-0.841	0.221	0.202
LPA	-0.599	0.454	-0.119
NEE	0.194	-0.544	0.714
NGE	0.631	0.108	0.406
LAG	-0.722	-0.167	-0.243
LOG	-0.441	0.221	0.177
PMG	-0.854	-0.110	-0.078
DC	-0.819	0.022	0.464
DEP	-0.754	0.108	0.480

The dendrogram (Figure 2) showed a combination of three groups for the six-rowed barley.
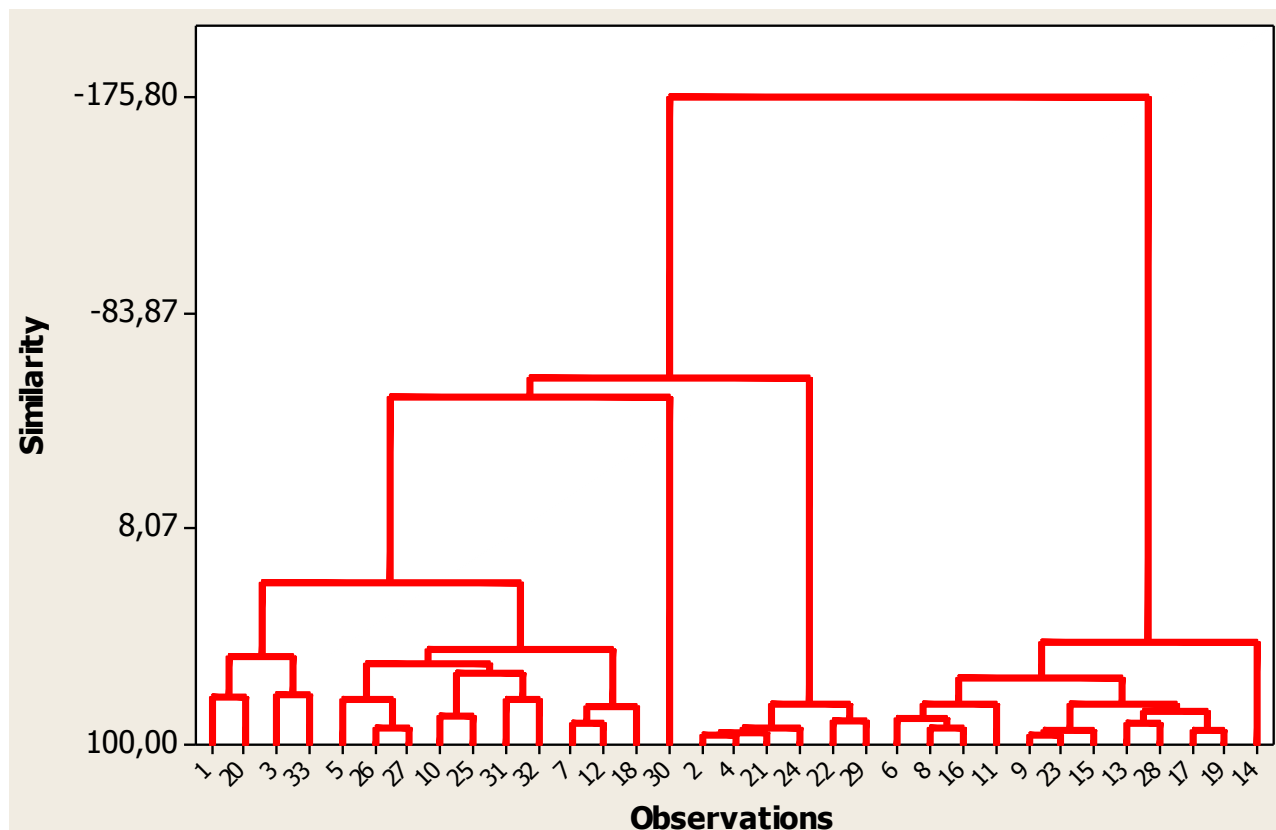
Figure 2. Dendrogram of 33 barley genotypes based on twelve characters.

significant correlations. All the following characters are correlated between themselves: HPL, LBA, PMG and LAG. Days to heading and days to maturity were correlated positively and significantly with LBA, PMG and LPA but significantly and negatively with NGE. The grain width and the grain length were correlated positively with days to maturity. A positive and very highly significant correlation was registered between HPL and HEP. NGE and PMG were highly correlated by negatively. A positive and significant correlation existed between LPA and PMG. There were not significant correlations between the grain protein content and all other characters. It was the same with the lower lemma length.

Principal component analysis was performed based on twelve characters. There were three Eigen values greater than 1, which determined the choice of the three components (Table 4). First component accounted for 41.05%; the second one for 15.5% and the third one for 11.73%. The first component was associated with: PMG, LBA, DC, DEP, LAG, NGE, HPL, LPA and LOG. The second component was associated with NTE and HEP. The NEE contributed to the third component.

In qualitative traits, the most variation among the genotypes concerned the growth habit, the curvature of the first rachis segment and the lower leaf sheath hairiness.

DISCUSSION

The study showed the existence of a high genetic variability and a great morphological distinction between the various genotypes for all quantitative traits analyzed. The diversity could be explored as a potential source of traits for crop improvement. A wide variation among genotypes of North Africa's barley was shown for morphological traits (Ben Naceur et al., 2012). Ahmadizadeh et al. (2011) studying genetic diversity of durum wheat landraces from Iran and Azerbaijan reported highly significant differences among genotypes in all of the morphological traits. Compared with four controls, much of traditional genotypes showed better yield components like that the 1000 grain weight, the tiller spike number per plant, the grain number per spike and also for the protein grain content and are therefore interesting for breeding programs.

The most important characters contributing to the variability were: PMG, LBA, DEP, DC, LAG, NGE, HPL, LPA and LOG which explained 41.05% of variation. NTE, HEP and NEE also explain an important part of variability (with cumulative % of components 2 and 3 of 27.23%). These results agree much with those found by Drikvand et al. (2012) were traits contributing to the most variance concerned awn length, plant height, grain yield, grain

number per spike, peduncle length, spike length and 1000 grain weight. Many of these traits have also contributed to the genetic differentiation among accessions of barley at Ambo (Setotaw et al., 2010).

The dendrogram of the evaluated barley genotypes divided all six-rowed barley studied into three cluster groups. In a work of Konichi et al. (1993), six-row barley genotypes investigated were also classified into three groups. It is the same for a study by Dimitrova-Doneva et al. (2014).

The first cluster included genotypes: 6, 8, 16, 11, 9, 23, 15, 13, 28, 17 and 19 characterized by long cycle, high values of 1000 grain weight and high means values of awn length. The second cluster grouped the genotypes: 2, 4, 21, 24, 22 and 29 which were characterized by the less large grains, short heading and cycle, short awns, short plants but by the high values of grain number per spike. The third cluster consisted of: 1, 20, 3, 33, 5, 26, 27, 10, 25, 31, 32, 7, 12, 18 and 30 which are characterized by the highest values of late and generally with the higher values of grain number per spike and spikelet number per spike.

The genotypes with long cycle have the greatest weight of 1000 grain and the highest stems. These results are consistent with those found by several authors (Bouzerzour and Monneveux, 1992; Al-Tabbal and Fraihat, 2012). The 1000 grain weight is very highly correlated but negatively with the grain number per spike. A negative significant correlation between these characters was also found by Žáková and Benková (2004) and Babaiy et al. (2011). The spike length was positively correlated (very highly significant) to plant height. Muhammad et al. (2012) found also a significant positive correlation between these two characters. It was also correlated to days to heading and days to maturity (positively and significantly). Mohtashami (2015) found a positive and significant correlation between the spike length and days to heading but positive and not significant correlation with days to maturity. The number of fertile tillers per plant was positively correlated to the spike length. Babaiy et al. (2011) showed a high positive correlation between these traits. The peduncle length was positively correlated with the 1000 grain weight. Babaiy et al. (2011) also found a same result. The awn length was very highly and positively correlated with 1000 grain weight and with days to heading and days to maturity. In fact, late genotypes have longer awns and heavier grains. Indeed, the role played by the awns in the drought resistance and in the grain filling was reported by several authors (Bort et al., 1994; Merah and Monneveux, 2014).

The largest value in grain protein content was 12.12% and concerned the Genotype 17 from Touggourt. The lowest value was 8.53% (Genotype 13 from El Bayadh). The level of protein in barley is highly variable, ranging from 7 to 25% according to a large USDA study involving over 10 000 genotypes (Ullrich, 2002).

On qualitative traits, the homogeneous characters within individuals of the same genotype were: growth habit, lower leaf sheath hairiness, shape of spike, rachilla hair type and shape of lemma type in sterile spikelet. Those with a little fluctuation were: pigmentation of awn tips, lemma type, disposition of lodicules in the fertile grain and glume length compared to grain. A large fluctuation within individuals of the same genotype concerned curvature of first rachis segment and spike density. The port of flag leaf and the lemma awn barb have moderately fluctuated between individuals of the same genotype. These results are consistent with the conclusions drawn by Argüello (1991). The characters which varied more among genotypes were: the growth habit, the curvature of the first rachis segment and the lower leaf sheath hairiness. In a study taken on wheat by Malik et al. (2014), the growth habit was also found as an important phenotypic marker for genetic variability and germplasm evaluation. For the lemma awn barb, proportion of rough awn was greater than the smooth awn. A similar result was found by Derbew et al. (2013).

These descriptors constitute a substantial database on an unknown germplasm and should be considered for breeding programs. The confirmation of these results and the study of other agronomic parameters and those related to the biotic and abiotic stresses will be used for better identifying this germplasm and better directing works of genetic improvement.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Characterization of accidents involving tractors in Brazilian federal highways in the state of Minas Gerais

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Minas Gerais state has the largest road network within Brazil on which thousands of vehicles travel daily. Due the increasing in the number of tractors in Brazil, traffic incidents may have also increased. Therefore, the present work aimed to characterize accidents involving tractors in Minas Gerais federal highways through the following indicators, event type, crash causes, time of the day in which the episode occurred, drivers age, climatic conditions and the road the case happened by analyzing Traffic Accident Reports (TAR). The incidents' information was provided by the Federal Highway Police (FHP) through TAR questionnaires. The total data set in the present work is composed by 148 TAR forms in Minas Gerais State between January, 2008 and September, 2011. The results were submitted to the chi-square test at 95% probability. The events' profile indicates collision as the main crash type, lack of attention as the main cause and the time period that has the most occurrences was 09:00 to 11:59 time period. Additionally, the group aged 25 to 30 years old were involved in the highest number of episodes, in good climatic conditions and the Brazilian Federal road BR-381 had the most evidenced cases.

Key words: Highway safety, tractor accident, collisions, safety.

INTRODUCTION

Minas Gerais state has the largest road network within Brazil (Minas Gerais, 2014), on which thousands of vehicles travel daily. Due the increasing in the number of tractors in Brazil, they are usually observed on public roads, especially closer to either farms or rural areas. Ericson (2010) argues that on a road in Cambodia, from the total amount of vehicles, approximately, 9% are tractors. However, the increased number of agricultural machinery on public roads may have also increased traffic incidents.

Regarding farm machinery episodes, studies have shown that there are some circumstances which may increase traffic risks. Road conditions (Costello et al., 2003; Peek-ASA et al., 2007), increased tractors number (Lehtola et al., 1994), time of day (Glascock et al., 1995; Gerberich et al., 1996), changes in seasons (Gkritza et

al., 2010), alcohol consumption (Gassend et al., 2009) are commonly found in literature as the main traffic risks.

Traffic accident analysis is need due the fact it allows the recognition of the events' main reasons which led the author to provide the best way to solve the problems. However, in order to analyse road traffic episodes, a data set containing the occurrence main characteristics is required (Debiase et al., 2004). In Denmark, Jorgensen (2008) made an accidents' database throughout 20 years of various rural sectors and argues that in years that awareness campaigns about deaths related with tractors were carried out, the total death numbers decreased when compared to the years the campaign was not publicized.

Therefore, the present work aimed to characterize accidents involving tractors in Minas Gerais federal highways through the following indicators, event type, crash causes, time of the day in which the episode occurred, drivers age, climatic conditions and the road the case happened by analyzing Traffic Accident Reports (TAR).

MATERIALS AND METHODS

The study was conducted in the Laboratory of Agricultural Machinery Accident Research in partnership with the 16th Federal Highway Police (FHP) Superintendence in Fortaleza, Ceará State, Brazil.

The incidents' information was provided by the FHP through Traffic Accident Reports (TAR). The TAR form is filled by the FHP after a car crash and, subsequently uploaded on to the FHP database. The gathered information is available to all regional offices of the country.

Each report contains the following information, the location (highway) where the event happened, machinery type (dozers, wheel tractor or mixed tractor) and weather condition. Additionally, also contains information about the episode type and cause, dead numbers (fatalities), the time period within the day the case happened and information about the driver.

In order to determine the minimum number of TAR forms required for statistical analysis, the operational characteristics curves methodology was utilized (HINES et al., 2006), and it is expressed in the following equation:

$$d = \frac{|\mu - \mu_0|}{\sigma} \qquad (1)$$

Where, d = number of samples; μ = population mean; μo = sample mean, and σ = standard deviation.

From the obtained d value, is possible to find the minimum amount of TAR forms required in the operational characteristics curves graphic (Montgomery and Runger, 2013). In the present study case, the graph information shows the minimum reports amount is 15.

The total data set in the present work is composed by 148 TAR forms in Minas Gerais State between January, 2008 to September, 2011. The different accident types filled in the reports and contained in this study were as follows, rear collision, side collision, transverse collision, collision with a fixed object, possible damages, running over, runway excursion, rollover and fire. The different incident causes reported were as follows, lack of attention, mechanical failure, driver fell asleep, incompatible speed for the road, alcohol intake and the safe distance to the tractor was not

kept. The time period within the day the crash occurred was subdivided in 8 classes each one having a 3 hours period and is presented in 0 to 24 h model. The classes are as follows, 00:00 - 02:59, 03:00 - 05:59, 06:00 - 08:59, 09:00 - 11:59, 12:00 - 14:59, 15:00 - 17:59, 18:00 - 20:59, 21:00 - 23:59.

Regarding to the operator's age, the first class of operator's age is between 0 and 19 years old. Subsequent classes have a 5-year-period starting at the age 20 and finishing at the age 60. The last class is above 60 years old. For climatic conditions analysis, the indicators are good (open sky), medium (cloudy) and bad (rain) environments. Concerning to event location, Brazilian federal highways are named as follows, firstly there is the BR prefix followed by three numbers. In Minas Gerais state, highways that had episodes reported were: BR-381, BR-262, BR-116, BR-153, BR-040, BR-365, BR-050, BR-135, BR-251, BR-267, BR-459, BR-354, BR-356, BR-452, BR-146, BR-460 and BR-474.

The results were submitted to a nonparametric statistical analysis by frequency distribution. The chi-square adherence test is used to find out which classes have significant difference between its indicators (Equation 2).

$$\chi^2 = \Sigma\left[\frac{(|Fo - Fe|)^2}{Fe}\right] \qquad (2)$$

χ^2 = Qui-square calculated; Fo = Observed frequency, and Fe = expected frequency.

The observed frequency relates to the number of registered occurrences. The expected frequency relates to the estimated value considering the total number of both accidents and indicators. The freedom degree corresponds to the total indicators' number minus one. Consequently, the observed value was compared with the expected tabulated value. When the observed value is higher than the tabulated value means there is significant difference.

RESULTS AND DISCUSSION

The chi-square adherence test (Table 1) made it possible to check which classes had significant difference between its indicators.

All the indicators were statistically significant at 95% probability which means incidents are not occurring randomly and all the cited classes may contribute for the event occurrence (Table 1). The reports have no information whether car drivers or tractor drivers caused the crash, therefore, either the car driver was the responsible or the tractor operator was the responsible or both car and tractor drivers were responsible.

By analyzing the cases, the most frequent episode type was collisions, all together (rear, side, and transverse collision as well as collision with a fixed object) they account for 75.68% of total accidents. Rear collision (28.28%), side collision (22.9%) and transverse collision (15.54%) were the most evidenced incident types (Figure 1).

Literature recognizes collisions as a serious problem and authors argue is the main type of tractor occurences on public roads in rural areas of India (Kumar et al., 1998), in Portugal (Justino, 2009) and the United States (Gkritza et al., 2010). This could be explained by the speed difference between both domestic cars and tractors on roads. For instance, the rear collision occurs

Table 1. Chi-square adherence test for accident indicators.

Indicators	x^2 Cal	x^2 Tab
Type	144.85*	16.92
Cause	134.55*	11.07
Highway	187.41*	26.29
Age group	20.18*	18.31
Time of day	69.73*	14,06
Weather conditions	157.39*	5.99

X^2 tab: chi-square tabulated 5%; X^2 cal: chi-square calculated; * (p<0.05); [NS] (not significant).
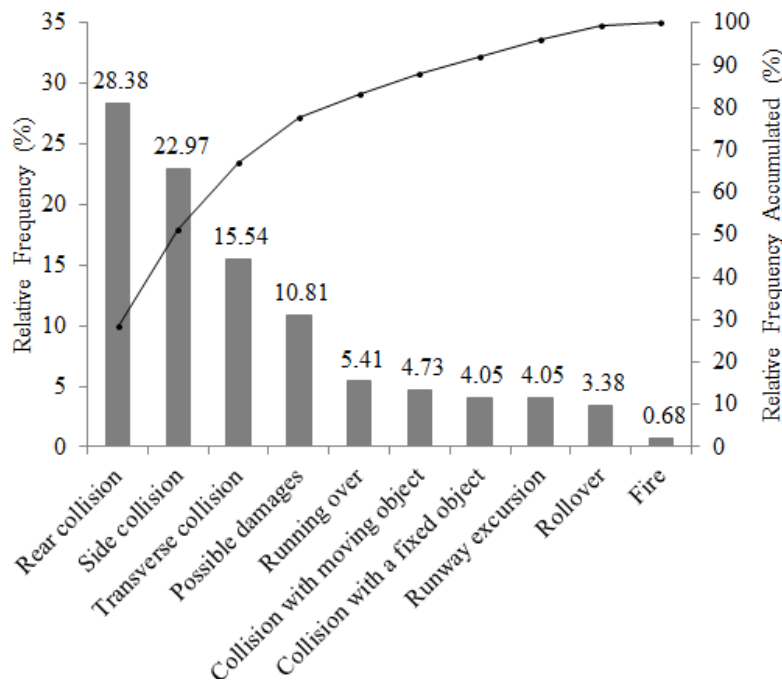
Figure 1. Crash types and their frequencies occurred on federal highways in Minas Gerais.

when a vehicle collides with the rear of the tractor, these cases can be associated with either a low speed tractor or drivers' lack of attention or the unfamiliarity of drivers with tractors on public roads. Welch (2006) states that rear collision occurs because the driver does not see the tractor and has no sufficient time to avoid the encounter.

Regarding to events' causes, the main reported cause was lack of attention (61.36%), followed by mechanical failure (18.18%) (Figure 2).

Schlosser et al. (2002) and Bunn et al. (2005) have also verified lack of attention as the main cause of accidents involving tractors. The lack of attention is usually attributed to human error, therefore the operator needs to have a special attention in order to reduce the general amount of incidents (Fernandes et al., 2011).

Concerning to the age of the injured, the group aged 25-30 years old suffered the highest number of crashes (16.18%), followed by the 35-39 group (13.24%) and the 45-49 set (12.5%) (Figure 3). The group with the most occurrences has less than 30 years old, mostly young workers having either a few experiences or no experience at all. On this account, their susceptible to accidents was higher. The results differ from Kumar and Dewangan (2009), who state a higher incidents' percentage for the 40-49 years group which was attributed to their physiological conditions.

Related with episodes' location, the highway when incidents were more evidenced was the BR-381 (26.35%), followed by BR-262 (13.51%) and the BR-116 (11.49%) (Figure 4). The Brazilian Federal road BR-381
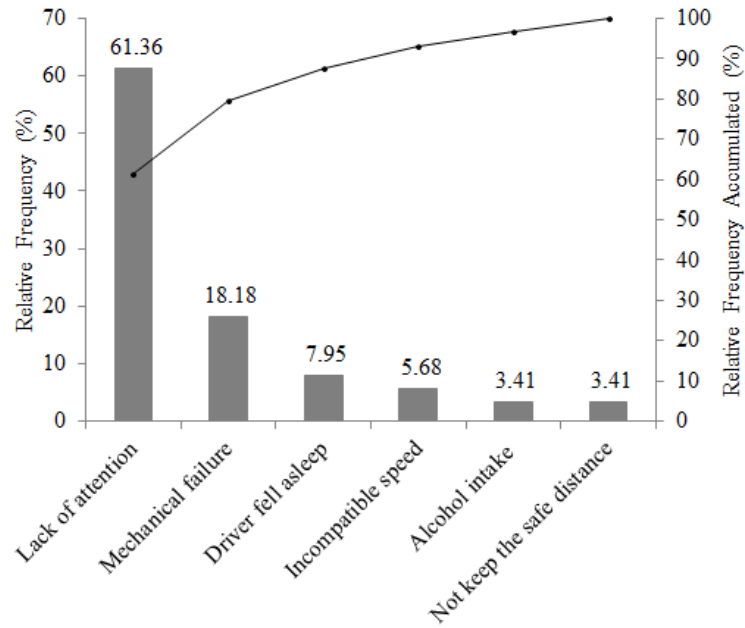
Figure 2. Causes and frequencies of episodes occurred on federal highways in Minas Gerais.
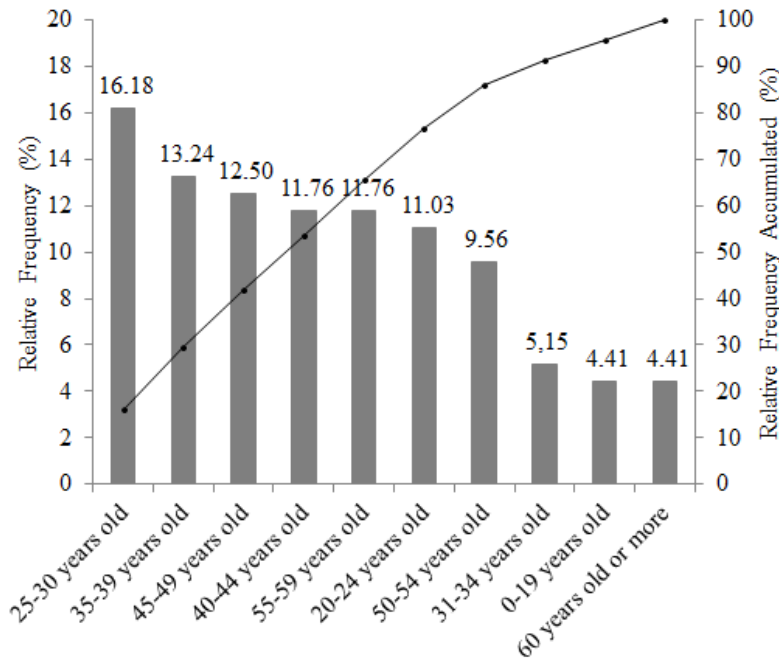
Figure 3. Operators' age groups involved in crash events on federal highways in Minas Gerais.

is the most extensive highway within Minas Gerais State which corroborates to explain the highest amount of occurrences happened in this road.

About to the time period within the day, the time period

that has the most occurrences number was 09:00 to 11:59 time period (24.32%), followed by 15:00 to 17:59 (22.30%) and 12:00 to 14:59, respectively (Figure 5).

The number of crashes was the greatest in the 09:00 to

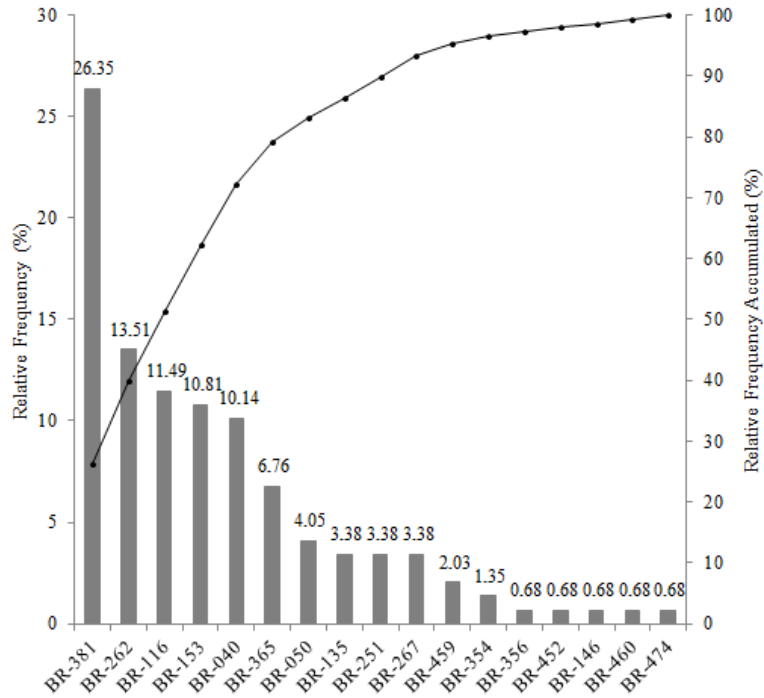**Figure 4.** Federal highways in Minas Gerais and their incidents frequency.
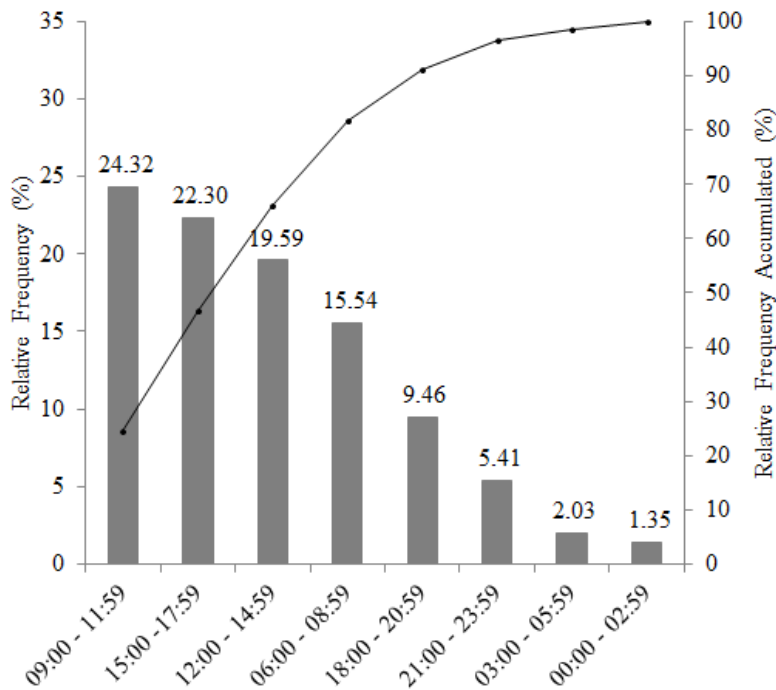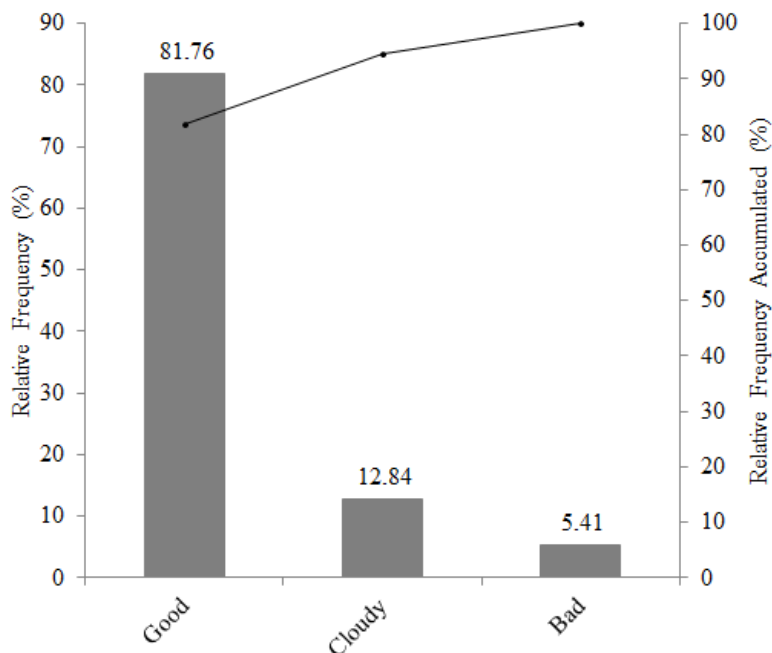

Figure 5. Time of day the cases happened in Brazilian federal highways, Minas Gerais State.

11:59 period. This data may be explained due the fact by this time period Brazilian operators finish the first work shift, usually having his attention decreased which could increase incident risks. Additionally, is important to

Figure 6. Evaluation of weather conditions on federal highways at the incident time.

highlight the accidents majority happened in daytime period, bearing in mind in Brazil the sun rises at 6a.m and sets at 6 pm, which has not influenced operator's visibility. Macedo (2015) argues that the bulk of incidents' events happen in both the afternoon and the morning, in this sequence, highlighting the crashes that happen when the operators' shift is almost finished, event usually due tiredness.

The weather conditions at the moment the episode happened is illustrated in Figure 5. Is possible to realize the incidents percentage was higher in good climatic conditions (81.76%) which means the operator's visibility had no interference at all (Figure 6).

Considering the events characteristics (70% of the cases happened due lack of attention and 80% happened in good both climatic and luminosity conditions) is possible to argue the incidents were more influenced by operators conditions (physical and psychological conditions) than weather conditions. Therefore, the operator needs to have a special attention in order to reduce the general amount of episodes.

Conclusion

The events' profile indicates collision as the main accident type, lack of attention as the main cause and the time period that has the most occurrences was 09:00 - 11:59 time period. The group aged 25-30 years old was involved in the highest number of episodes, in good climatic conditions and the Brazilian F ederal road BR-381 had the most cases probably due its extension. Operators must have short-time breaks within the tractor operation in order to improve attention. It is not advisable to operate the machinery in roads the tractor speed is not suitable for.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Anaerobic biodigestion in Indian batch-type biodigester, using poultry litter as substrate for the production of biogas

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Poultry litter is being produced in large quantities due to the accelerating growth of the broiler industry in recent years, which implies a greater energy dependence and cost of these systems. This study seeks to evaluate the capacity of an anaerobic batch biodigester to produce biogas from poultry litter. To this end, three tests were conducted: 1st: Poultry Litter + Water - [PL + W]1, 2nd: Poultry Litter + Water - [PL + W]2. 3rd: Description of treatments: Period of implementation: September 2013 to March 2014. T1 - Poultry Litter + Biofertilizer + Water, (PL+B+W) - of which: 28.25 kg of water + 28.25 kg of biofertilizer + 3.5 kg of litter. T2 - Poultry Litter + Biofertilizer (PL+B) - of which: 56.5 kg of biofertilizer + 3.5 kg of litter. T3 - Poultry Litter + Water (PL+W) - of which: 56.5 kg of water + 3.5 kg of litter. The results showed that the anaerobic biodigestion process was efficient in producing biogas in test 3, and that the three evaluated treatments produced different volumes of biogas, with the best treatment being the one that used a poultry litter associated with biofertilizer (T2 - PL+B), suggesting that the biofertilizer acted as a system that enabled the process, followed by the treatment that used poultry litter associated with biofertilizer and water (T1 - PL+B+W). The lowest values were observed in the third treatment, which used a mixture of poultry litter and water (T3 - PL+W).

Key words: Biogas, poultry, waste, biomass.

INTRODUCTION

The Brazilian broiler industry had an output of about 8 million tons of meat in 2014, which corresponds to 16.4% of world production. With this, exports reached 1.8 million tons, increasing Brazil's share to almost a third of total

foreign trade, with emphasis on the quantity of shipped cuts. The consumption of 35.1 kg of poultry per inhabitant per year is among the highest observed in developed countries (Anualpec, 2014).

As in other agricultural activities, broiler production generates a very large amount of waste (poultry litter and dead birds), which, if well managed, can not only become an important source of income and add value to the activity, but also turn into a model of sustainable production, which is increasingly becoming a market demand. To this end, a system will need to be adopted for the treatment of this waste in order to avoid possible contamination of the environment (Girotto and Ávila, 2003; Güngör-Demirci and Demirer, 2004; Angonese et al., 2006).

The biogas produced from the biodigestion of poultry litter can be used to heat the chicks by using equipment that burns the biogas and, consequently, produces heat, which is essential for the survival of these animals in the first two weeks of life. It can also replace electrical power in, for example: Lighting (lamps); water heating (for the sterilization of equipment, washing facilities, showers, etc.); stoves; grain milling, etc. (Silva et al., 2005).

Anaerobic biodigestion is a treatment system in which biomass is degraded to form methane (CH_4) and carbon dioxide (CO_2) in anaerobic conditions (Demirer and Chen, 2005). The methane produced can be used as a source of energy, replacing fossil fuels and thus adding value to production and decreasing the emission of carbon dioxide (Silva et al., 2005; Orrico et al., 2007; Santos et al., 2007). The advantages of the process are: Reduction of pathogenic micro-organisms and odors; occupation of a small physical space for the treatment of waste; and the easy control of the released gases or effluents from the process. In anaerobic processes, or in anaerobic biodigestion systems, the degradation of organic matter involves the activity of optional and required prokaryotic micro-organisms, whose species belong to the group of hydrolytic-fermentative bacteria, acetogenic bacteria that produce hydrogen and methanogenic archaea (Côté et al., 2006; Alvarez et al., 2006).

For the digestion of biomass from animal waste to be economically attractive, the physical and chemical properties of the waste must be compatible with the considered biodigester design. Thus, it is important to understand the principles of operation of most biodigestors to help in the selection and planning of the treatment model based on anaerobic biodigestion. The importance of this knowledge is related to the elevated production of methane and the rates of biogas production, which are dependent on the relative contribution of the waste and biodigestor costs in the final costs of the biogas (Santos, 2001; Nishimura, 2009).

In environmental terms, the use of biogas represents an overall improvement in process efficiency. Since biogas is usually a residue of the decomposition process of organic matter, the benefits attributed to it are linked to

its intended use. The two main alternatives for the energetic exploitation of biogas are the conversion to electrical energy and its thermal use (Fisher et al., 1979; Alvarez et al., 2006).

Biogas emissions into the atmosphere have negative impacts on the environment and society since it contributes to the further intensification of the greenhouse effect through methane emissions into the atmosphere. In addition, it causes unpleasant odors through the emission of putrid and toxic gases due to the concentration of sulfur compounds in the gas, in addition to a small, but not negligible presence of bacteria responsible for the anaerobic digestion of organic waste (Costa, 2006). When converted into electrical energy, the advantages of the use of biogas are related to the avoided emissions through the generation of electrical power using a renewable source, the efficiency of conversion systems and the reduction of dependence on energy from the network, reducing local overload (Oliveira and Ribeiro, 2006).

Biogas has several applications in energy. Although its main application is as a fuel in an internal gas combustion engine that powers an electric generator, it can be directed to other purposes. Among these other applications, one could highlight the use of biogas in gas-fired heaters for the production of hot water for environmental conditioning or heat processing, in the drying of grain in rural properties, in the drying of sludge at waste water treatment stations, in the burning in boilers, in the heating of pig farms, in vehicular use, and in gas lighting, among others (Pecora, 2006). Biogas is composed, for the most part, by two gases: methane, which is its energy constituent, and carbon dioxide, since about 95% of the volume is composed of these two gases (Silva et al., 2005).

The harnessing of energy from biogas does not only contribute to the preservation of the environment, it also brings benefits to society by promoting the use or reuse of "disposable" and/or low-cost resources, by reducing our dependence on fossil fuels through a greater variety in fuels, by enabling the generation of decentralized power, by increasing the supply of power, by enabling the generation of local jobs, by reducing odors and toxins in the air, by reducing the emission of pollutants through the substitution of fossil fuels, by helping in making Landfill and Waste Treatment plants economically viable, by optimizing the use of local resources, and by increasing the viability of basic sanitation in the country, enabling the technological development of Sanitation and Energy companies (Ross et al., 1996). In this context, this study evaluated the capacity of an anaerobic batch biodigester to produce biogas from poultry litter.

MATERIALS AND METHODS

This study followed the methodology described in Caetano (1991) and adapted it to this work, in which a batch-type biodigester with a

Table 1. Yield at every 7 days (m^3/kg of biomass) of biogas for the batch-type biodigester filled with poultry litter and with the addition of water.

RTD*	[PL+W]1	[PL+W]2
0	0	0
7	0.0123	0.0093
14	0.0172	0.0134
21	0.0234	0.0212

*Retention time in days.

capacity of 60 L was used, filled one time for each one of the experiments, keeping it in fermentation for the desired period, with the material being discharged after the end of the effective period of biogas production. The biodigester was developed by LACTEC - Institute of Technology for the Development of Paraná - Curitiba - Paraná - Brazil, and was intended for teaching purposes. It was adapted for the application of the methodology of this study.

The poultry litter was obtained from a conventional chicken production barn of 1,200 m^2 installed in a rural property located at the geographic coordinates 25° 44' 06'' S and 53° 04' 52'' W in the municipality of Dois Vizinhos - Paraná - Brazil. Three batches of litter from chickens reared for 40 days, on average, were used.

The tests were carried out between the months of May 2012 and March of 2014, with a minimum temperature of 15°C and a maximum of 37°C, according to Simepar. During this period, the following tests were performed: 1st test: Poultry Litter + Water - [PL + W]1. Implementation period: May to September to November 2012. 30 Kg PL + 30 kg W. 2nd test: Poultry Litter + Water - [PL + W]2. Period of implementation: October 2012 to March 2013. 15 kg PL + 45 kg W. 3rd test: Description of treatments: Period of implementation: September 2013 to March 2014. T1 - Poultry Litter + Biofertilizer + Water, (PL+B+W) - of which: 28.25 kg of water + 28.25 kg of biofertilizer + 3.5 kg of litter. T2 - Poultry Litter + Biofertilizer (PL+B) - of which: 56.5 of biofertilizer + 3.5 kg of litter. T3 - Poultry Litter + Water (PL+W) - of which: 56.5 of water + 3.5 kg of litter.

The experiments were carried out for a period of 56 days and the volumes of biogas produced were observed by means of a piston every 7 days, recording the values and quantifying the average biogas produced.

The potential for biogas yields was calculated using the total production data and the quantities of material *in natura*. The values were expressed in cubic meters of biogas per kg of substrate used (m^3/kg).

RESULTS AND DISCUSSION

The obtained results showed that the two models sized and characterized as tests [PL+W]1 and [PL+W]2 proved to be unviable from the point of view of the biogas production process. This data can be seen in Table 1.

One can observe through these tests that anaerobic digestion proved to be slow at the beginning for the production of biogas, thus presenting a lag phase that was too long, exceeding 15 days. These two batches were therefore shut down after 21 days, with yields of 0.0234 and 0.0212 m^3 of biogas with the treatments [PL+W]1 and [PL+W]2, respectively. It should be noted

that these trials only had two repetitions, which means no statistical program could be applied to them.

The test characterized as number 3 proved viable and capable of being used as a model in the use of biomass that is basically made up of poultry litter. The discussion of this work is therefore based on this trial, in which three characteristic treatments were adopted. The average potential of biogas yields in trial 3 are presented in Table 2, in m^3 of biogas per kg of dry matter.

According to the results obtained in Table 2 and shown in Figure 1, higher yields of biogas can be observed when the digester is filled with poultry litter + biofertilizer when compared to digesters filled with poultry litter + biofertilizer + water and poultry litter + water. The mixture that produced the least biogas was the mixture poultry litter + water mixture (T-3). This behavior was observed during the entire process.

When the means obtained in the three treatments are compared, one can observe that treatment T2 proved to be superior in the production of biogas, at the level of 5% through the Tukey test, compared to the two other treatment methods (T1 and T3) for the period under evaluation, since the mean values were 0.4106±0.0128, 0.3264±0.0243 and 0.0804±0.0023 m^3 of biogas per kg of biomass added to the process for each one of the assessments, respectively. The data relating to the volumetric biogas yields are presented in Figures 1 and 2.

Considering the results obtained with the treatments, one can see that the treatment PL+B produced a greater quantity of biogas during the 56 days and showed a peak of biogas production at 42 days with a drop in production after 49 days, a drop in production that lasted until the end of the process. At the end of the process this treatment presented a kinetics equal to $y = -0.0004x^2 + 0.0312x -0.159$ with a correlation coefficient $R^2 = 0.9641$.

It should be pointed out that the time variable was important in this analysis, since the volumes of produced biogas increased over time. This becomes important because an understanding of the range with the highest biogas yields in a given time period is crucial for the dimensioning of the utilization of the generated biogas.

One can see that the other two treatments showed the same behavior, but with production starting a little later and with the treatment PL+B+W only reaching the volumes recorded in the treatment PL+B after around 14 days. As such, the kinetics regarding treatments PL+B+W and PL+W, which represent the behavior of each treatment and are expressed through polygonal adjustments curves, were $y = -0.0004x^2 + 0.0312x -0.159$ with $R^2 = 0.9641$ and $y = -0.0002x^2 + 0.0142x -0.0904$ with $R^2 = 0.7944$, respectively. The treatment that produced the lowest amount of biogas was PL+W.

The determination coefficient R^2 is the percentage of the variation in the dependent variable explained by the independent variable. It should be clarified that the closer R^2 is to 1.0, the lower the difference between the actual

Table 2. Yield at every 7 days (m³/kg of biomass) of biogas for the batch-type biodigester filled with poultry litter and diluted with biofertilizer and water.

RTD[1]	PL+B+W	PL+B	PL+W
7	0.0482	0.1242	0.0161
14	0.1684	0.1875	0.0183
21	0.3682	0.3745	0.1285
28	0.3823	0.5289	0.1546
35	0.4230	0.5529	0.1573
42	0.4554	0.5593	0.0862
49	0.4420	0.5293	0.0558
56	0.3251	0.4286	0.0265
Mean[2]	0,3264±0,0243A	0,4106±0,0128B	0.0804±0.0023C
Total for the period[3]	2,611	3,284	0,643

[1] Retention time in days. [2]Means followed by the same letter horizontally do not differ by Tukey's Test at the level of significance of 5%. [3] m³ of biogas/kg of biomass.
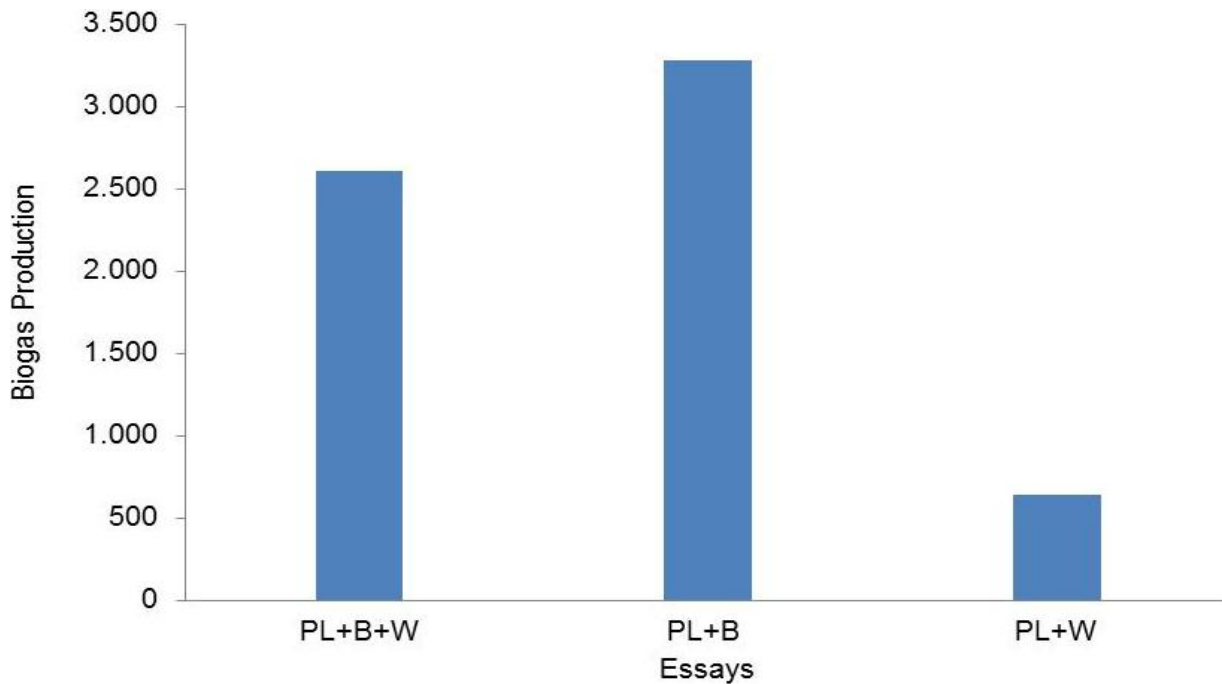
Figure 1. Cumulative biogas yields for each treatment during the process.

data and the points on the behavior fitting curve or the kinetic behavior of the variable.

In a general analysis regarding the total values for biogas yields, one can see that the volumes of the three treatments reached 2,611.20 + 3,284.80 + 0643.20 m³ for PL+B+W, PL+B and PL+W, respectively, totaling approximately 6,540 m³.

When calculating the percentages of each of the treatments in relation to the total volume of biogas produced in the three experiments, one sees that the treatment PL+B+W produced 40%, the treatment PL+B

produced 50%, and treatment PL+W produced 10% of the biogas.

The values point to the influence of the period on the yield potential for biogas found in this experiment and is similar to the studies by Ortolani et al. (1991) who found highly significant differences for the biogas yield potential means between three tests.

Through the visualization of the behavior in the fitting curves related to the volumes of biogas of the treatments, it is possible to plan a system that meets a certain energy demand. The anticipation of peak biogas production in
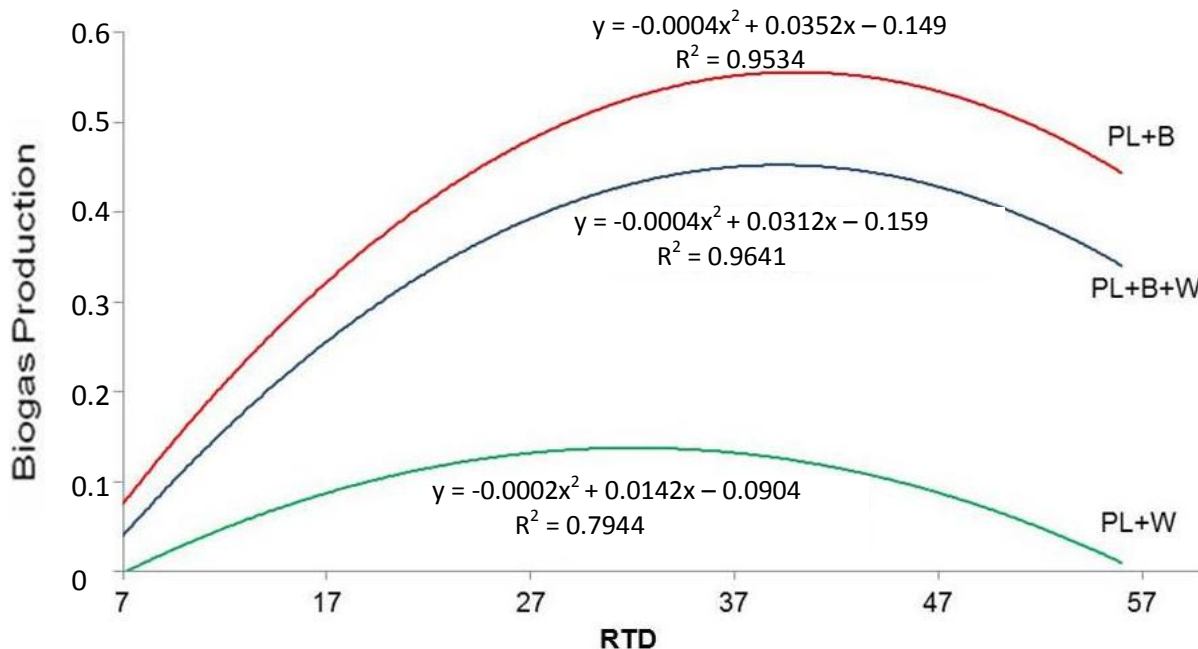
Figure 2. Kinetics of the second-degree polynomial behavior of the systems during the process. Equations are followed by the determination coefficient for each treatment R^2.

treatment PL+B can be clearly seen in Table 2. This is probably due to the addition of biofertilizer in association with the poultry litter, accelerating the stages of anaerobic biodigestion and increasing the speed of biogas production.

In order to plan the production of biogas for power generation, the areas with the most expressive biogas production in the curves should be considered so as to prevent a lack of energy production when the demand for it is high. For example, proper planning should be used when using anaerobic biodigestion of poultry litter for the production of biogas, taking into account the stages of higher biogas production in relation to the batches of poultry, thus increasing the viability of the biodigester and the generation of energy.

Under these conditions, it is clear that the best treatment was the one that used poultry litter associated with biofertilizer, followed by the treatment that used poultry litter associated with biofertilizer and water. The lowest values were observed in the third treatment, which used a mixture of poultry litter and water.

It should be noted that the addition of poultry litter to anearobic digestion reduces the conversion efficiency of other bird waste products into biogas. Some authors, however, have reported high potential for the production of biogas. In this respect, Webb and Hawkes (1985) operated a biodigester with poultry litter (manure + sawdust) and observed a production of biogas which yielded 0.245 to 0.372 m³ of biogas per kg of added biomass. These values are similar to those obtained in this work.

Magbanua Junior et al. (2001) tested anaerobic digestion using poultry waste supplemented with biofertilizers from pigs and cattle in various proportions. They concluded that the waste that received the biofertilizers from pigs, cattle and birds together produced more biogas compared with the waste (poultry litter) from birds alone. These data are in alignment with the data obtained here, which showed the same behavior. This behavior is based on the use of micro-organisms present in these different materials, which act as inoculants that activate the system more rapidly.

It should be emphasized that the biogas is formed by a mixture of gases produced during the fermentation process. The main gaseous components of the biogas are methane and carbon dioxide. Methane is an excellent fuel and the greater the methane content, the purer the biogas (Magalhães, 1986; Santos, 1992; Sanchez et al., 2001).

Given that poultry litter is produced at time intervals, that is, its availability is not continuous due to the mode of production, and considering its physical and chemical characteristics, such as a high solid content, low moisture and particle size, the ideal biodigester for its anaerobic digestion is a batch-type biodigester, which may be managed in the battery form or sequentially. The disadvantage of the management in battery form is related to the speed of fermentation of the litter, which is slow, making the harnessing of the biogas more difficult (Magellan, 1986; Santos, 2001).

It may be necessary for the poultry litter to undergo a pre-treatment before being added to the biodigester, the

most recommended would be a grinding step since the wood shavings may be too large and this can reduce the efficiency of the micro-organisms. Looking at the moisture content of the litter, it is necessary to add water to decrease the solid content and dilute its content (Santos, 1992, 2001).

The function of the inoculum is to speed up the process, mainly due to the high contents of cellulose and lignin, materials that are hard to digest and that are present in the litter. The inoculum may consist of already biofertilized manure from cattle, poultry, pigs, etc., which contains a large microbial flora of acetogenic and methanogenic bacteria that are fundamental for digestion (Santos, 1992; Steil, 2001; Zhu et al., 2004).

Conclusion

The anaerobic biodigestion process was efficient in producing biogas in test 3, and the three evaluated treatments produced different volumes of biogas, with the best treatment being the one that used poultry litter associated with biofertilizer (T2 - PL+B), suggesting that the biofertilizer acted as a system that enabled the process, followed by the treatment that used poultry litter associated with biofertilizer and water (T1 - PL + B + W). The lowest values were observed in the third treatment, which used a mixture of poultry litter and water (T3 - PL + W).

Conflicts of Interest

The authors declare there are no ethical, publishing of financial conflicts of interest regarding the data of this study.

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Full Length Research Paper

Energy potential of poultry litter for the production of biogas

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Poultry litter, a waste product from broiler farming, can be transformed into biogas when subjected to anaerobic biodigestion. This study proposes to evaluate the potential of poultry litter for energy generation in order to meet the energy demands of the poultry production chain in the southwest region of the state of Parana - Brazil, when used as a substrate for the production of biogas. Based on the volumes of biogas produced, the viability of biogas production was determined. The results reveal that the anaerobic biodigestion process was efficient in biogas production. It was also possible to determine that the use of poultry litter in biogas production is viable, since the raw material for its production is available in the entire southwest region of Paraná. The presentation of the three treatments proved to be important, because in the three systems the production of significant quantities of biogas was possible, constituting a potential replacement for non-renewable fuel sources derived from petroleum.

Key words: Biogas, energy, waste, biomass.

INTRODUCTION

As in any economic activity, the production chain in broiler farming generates industrial and rural sub-products that have environmental impacts. The inappropriate management of poultry waste affects the rivers and groundwater supplying both rural and urban environments, potentially causing ecological imbalances,

spreading pathogens and contaminating drinking water with ammonia, nitrates, phosphates and other toxic elements. Mitigating these risks is increasingly becoming a requirement for poultry farmers (Almeida and Navarro, 1997).

Since its activities involve high energy consumption

and waste production, the broiler industry can convert its waste into energy - as happens in the sugar-ethanol industry with the sugarcane vinasse and bagasse. The sub-product of poultry farming, the "poultry litter", is of extreme importance in the poultry production chain in the southwest region of Paraná - Brazil. Poultry litter is made up of a mixture of excretions (feces and urine) with the substrate used to receive and absorb the moisture from these excretions (wood shavings, sawdust, straws from cereals, chopped hay, etc.), feathers and skin flakes from the birds, and food remnants that fell from the feeders. The purpose of the poultry litter is to avoid direct contact of the bird with the floor, to serve as a substrate for the absorption of water and incorporation of feces and feathers, and to contribute to the reduction of temperature fluctuations in the shed. The poultry litter has a significant energy potential that can be made available as biogas through anaerobic biodigestion, contributing to the increased economic attractiveness of the activity (Rodrigues, 1997; Bellaver and Palhares, 2003).

Poultry litter is being produced in large quantities due to the accelerating growth of the broiler industry in recent years, based mainly on the development of shed technology, which implies a greater energy dependence and cost of these systems (Rocha et al., 2008).

In addition to the environmental impacts, the energy issue is another challenge of strategic importance to the world economy, one that directly affects the poultry production chain in the southwest region of Paraná - Brazil. In the poultry production chain, energy costs are highly significant, highlighting the great demand for energy in both the slaughter and processing units and in the chicken farms themselves (Fiorentin, 2005; Uba, 2014).

The biogas produced in bio-digesters consists essentially of 60 to 70% methane (CH_4) and 30 to 40% carbon dioxide (CO_2), in addition to traces of O_2, N_2, H_2S, etc., that for organic waste. In the biodigestion of fatty substances, the gas may contain up to 75% methane, and methane is the biogas component that has fuel properties, serving, for example, to power engines with a quite similar performance as those powered by natural gas or liquid fuels. The difference lies in the economic and environmental aspects, given that biogas is one of the by-products of the processing of organic waste, producing less solid sulfur-based waste (Lusk, 1998; Jonsson, 2004; Kapdi et al., 2005).

Methane has a calorific value of 9,100 kcal/m^3 at 15.5°C and 1 atm, its flammability occurs at mixtures of 5 to 15% with air. Biogas, on the other hand, has a calorific value ranging from 4,800 to 6,900 kcal/m^3 due to the presence of other gases than methane. In terms of energy equivalence, 1.33 to 1.87 and 1.5 to 2.1m^3 of biogas is equivalent to 1 L of gasoline and diesel, respectively. In comparison, natural gas is made up for 88% of methane (Kirb and Biljetina, 1987; Ross et al., 1996; Ferrer et al., 2004; Epe, 2007).

The potential for harnessing the energy from biogas depends, above all, on the economic viability of integrated projects for the production, collection and use of biogas. To assess the economic viability of projects, first the costs of investment, operation and maintenance for each specific project, in addition to the revenue obtained through the sale of energy or cost savings, need to be considered. In this case, the final use of the biogas is the determining factor, since all the economic parameters depend on the use of the fuel, be it for the production of heat, electricity, co-generation or simply for the sale of gas (Murphy et al., 2004; Persson et al., 2006).

Since the proposition is to provide a treatment system that has energy self-sufficiency in poultry farming as main advantage, an understanding of the energy consumption in this activity is essential for the proper planning of the treatment system so as to detect if it will be able to achieve self-sufficiency or simply contribute to a decline in the consumption of external power (Broughton et al., 1998).

As such, this work seeks to evaluate the energy potential of the poultry litter produced in the southwest region of Paraná used as substrate for the production of biogas.

MATERIALS AND METHODS

This study followed the methodology described in Caetano (1991) and adapted it to this work, in which the employed biodigester was of the Batch type with capacity of 60 L, which was filled one time in each of the experiments, keeping it in fermentation for the desired period, with the material being discharged after the end of the effective period of biogas production. The biodigester was developed by LACTEC - Institute of Technology for the Development of Paraná - Curitiba - Paraná - Brazil, and was intended for teaching purposes. It was adapted for the application of the methodology of this study.

The poultry litter was obtained from a conventional chicken production barn of 1,200 m^2 installed in a rural property located at the geographic coordinates 25° 44' 06'' S and 53° 04' 52'' W in the municipality of Dois Vizinhos - Paraná - Brazil. Three batches of litter from chickens reared for 40 days, on average, were used.

The substrate used to line the floor was *pinus* sawdust and the thickness of the initial litter was approximately 12 cm, a volume that increased with the placing of new substrates on top of the others for subsequent production cycles. For the purposes of the experiment, however, the litters of the third batch were used. One (01) Indian batch-type biodigester was filled. The biodigester was developed by LACTEC - Institute of Technology for the Development of Paraná - Curitiba - Paraná - Brazil, and was intended for teaching purposes. It was adapted for the application of the methodology of this study.

The tests were carried out between the months of May 2012 and March of 2014, with a minimum temperature of 15°C and a maximum of 37 °C, according to Simepar. During this period, the following tests were performed: *1st test:* Poultry Litter + Water - [PL + W]1. Implementation period: May to September to November 2012. *30 kg PL + 30 kg W. 2nd test:* Poultry Litter + Water - [PL + W]2. Period of implementation: October 2012 to March 2013. 15 Kg PL + 45 kg W. *3rd Test:* Description of treatments: Period of implementation: September 2013 to March 2014. T1 - Poultry Litter + Biofertilizer + Water, (PL+B+W) – of which: 28.25 kg of water +

28.25 kg of biofertilizer + 3.5 kg of litter. T2 - Poultry Litter + Biofertilizer (PL+B) - of which: 56.5 of biofertilizer + 3.5 kg of litter. T3 - Poultry Litter + Water (PL+W) - of which: 56.5 of water + 3.5 kg of litter.

The experiments were carried out for a period of 56 days and the volumes of biogas produced were observed by means of a piston every 7 days, recording the values and quantifying the average biogas produced.

Biogas production was quantified according to the observed accumulation in production, with monitoring being carried out daily and a reading being taken at least every 07 days.

The reading was taken by measuring the vertical displacement of the gasometer through a ruler fixed to the biodigester, located next to the bell. After each reading, the gas meters were zeroed using the biogas discharge valve.

The data was submitted to analysis of variance (ANOVA), and the means and standard deviations were calculated, through which it was possible to compare one data set at the same time. The means obtained for the three different treatments were compared by Tukey's test. A significance level of 5% ($P < 0.05$) was used for all analyses.

RESULTS AND DISCUSSION

The obtained results showed that the two models sized and characterized as tests [PL+W]1 and [PL+W]2 proved to be unviable from the point of view of the biogas production process. The test characterized as number 3 proved viable and capable of being used as a model in the use of biomass that is basically made up of poultry litter. The discussion of this work is therefore based on this trial, in which three characteristic treatments were adopted. According to the data obtained, higher yields of biogas can be observed when the digester is filled with poultry litter + biofertilizer when compared to digesters filled with poultry litter + biofertilizer + water and poultry litter + water. The mixture that produced the least biogas was the mixture poultry litter + water mixture (T-3). The average potential of biogas yields in trial 3 are presented in Table 1, in m^3 of biogas per kg of dry matter.

In a general analysis regarding the total values for biogas yields, one can see that the volumes of the three treatments reached 2,611.20 + 3,284.80 + 643.20 m^3 for PL+B+W, PL+B and PL+W, respectively, totaling approximately 6,540 m^3.

When calculating the percentages of each of the three treatments of the 3rd trial in relation to the total volume of biogas produced in the three experiments, one sees that the treatment PL+B+W produced 40%, the treatment PL+B produced 50%, and treatment PL+W produced 10% of the biogas. So considering that the three treatments evaluated in this study showed production of biogas, with treatment T2 being the most viable, producing a volume of biogas in the period of 3,284.80 m^3/kg of poultry litter biomass, it is possible to determine the energy capacity of the poultry litter produced in the southwest region of Paraná - Brazil.

Considering that the three scaled and monitored experiments produced values varying between 0.643 to 3.284 m^3/kg of poultry litter, the following calculations will

use the lowest values, taking into account that several variables can interfere with the biogas production process. To perform this calculation, the methodology described by Mahadevaswamy and Venkataraman (1986) was taken into account, where: *CPB= Mscf 0.643 $m^3$of biogas/kg of dry poultry litter matter.*

As such, a CPB *(Capacity for Producing Biogas)* = 0.643 m^3/kg of poultry litter for 51,219,177 kg of poultry litter in the 38 municipalities in the southwest region of Paraná / year (Amsop, 2014), will potentially yield a volume of 32,933,931 m^3/year in biogas. It should be noted that this is the lowest estimated value, considering only the lowest values of the performed trials.

If we were to consider the three treatments, the volume would be much higher, since the production of biogas in treatment 1 and 2 were significantly higher than those in treatment 3. These data can be seen in Table 2.

Taking into consideration the values presented in Table 2, and using the calorific value of the produced biogas, one can estimate the savings in the use of other fuels by using the equivalence of 0.61 L of gasoline, 0.58 L of kerosene, 0.55 L diesel oil, 0.45 kg of cooking gas, 1.5 kilos of wood, 0.79 L of hydrated ethanol and 1.43 kWh of electricity for each m^3 of biogas (Ferraz and Mariel, 1980; Sganzerla, 1983; Nogueira, 1986; Santos, 2000). These data can be seen in Table 3.

The data presented in Table 3 show the viability of using poultry litter for biogas production, since the raw material for its production is available in the entire southwest region of Paraná. The presentation of the three treatments proved to be important, because in the three systems the production of significant quantities of biogas was possible, constituting a potential replacement for non-renewable fuel sources derived from petroleum.

One can observe that it is possible to save up to 117,624,599 kWh of electrical energy; 132,880,509 liters of hydrated ethanol; 252,304,765 cubic meters of firewood; 75,691,429 kg of cooking gas, considering that each gas canister has a capacity of 13 kg of gas, this would imply savings of 5,822.41 gas bottles. In addition, the savings would be equivalent to 92,511,747 liters of diesel oil; 97,557,842 L of kerosene and 102,603,937 L of gasoline.

It is important to stress that there are several technologies for the conversion of biogas into other forms of energy. When there is a mixture of air and biogas in a process called controlled combustion, the chemical energy contained in the biogas molecules is converted into mechanical energy. The mechanical energy is converted into electrical energy through an alternator (Synchronous Generator).

Cogeneration (combined generation of heat and power) and trigeneration (combined generation of heat, electricity and cold) are important alternatives for the use of energy, since these conversion processes allow for the reuse of residual thermal energy, increasing energy efficiency. Generating electrical energy from biogas has advantages

Table 1. Yield at every 7 days (m^3/kg of biomass) of biogas for the batch-type biodigester filled with poultry litter and diluted with biofertilizer and water.

RTD[1]	PL+B+W	PL+B	PL+W
7	0.0482	0.1242	0.0161
14	0.1684	0.1875	0.0183
21	0.3682	0.3745	0.1285
28	0.3823	0.5289	0.1546
35	0.4230	0.5529	0.1573
42	0.4554	0.5593	0.0862
49	0.4420	0.5293	0.0558
56	0.3251	0.4286	0.0265
Mean[2]	0.3264±0.0243A	0,4106±0.0128B	0.0804±0.0023C
Total for the period[3]	2.611	3.284	0.643

[1] Retention time in days. * Means followed by the same letter horizontally do not differ by Tukey's Test at the level of significance of 5%. [3] m^3 of biogas/kg of biomass.

Table 2. Biogas production capacity of the southwest region.

Treatment	Total volumes (m^3/year)
T1	136,344,271
T2	168,203,177
T3	32,933,931

Table 3. Equivalent use of biogas in relation to other sources.

Fuel type	T1 - PL+B+W 136,344,271 m^3/year	T2 - PL+B 168,203,177 m^3/year	T3 - PL+W 32,933,931 m^3/year
Gasoline (L)	83,170,005	102,603,937	20,089,697
Kerosene (L)	79,079,677	97,557,842	19,101,679
Diesel Oil (L)	74,989,349	92,511,747	18,113,662
Cooking Gas (kg)	61,354,921	75,691,429	14,820,268
Wood (m^3)	204,516,406	252,304,765	49,400,896
Hydrated Ethanol (L)	107,711,974	132,880,509	26,017,805
Electricity (kWh)	95,345,644	117,624,599	23,030,720

from strategic, economic, environmental and social perspectives. Among these advantages, the following could be highlighted; (i) decentralized generation, which avoids investment in transmission due the proximity with charging points; (ii) use of cheap fuel (process waste) that is available on site; (iii) (renewable) biomass energy, low emission of pollutants, contributing to the mitigation of the greenhouse effect; and (iv) potential use of resources that were previously reserved for the payment of electrical power utilities, in actions for the social development of the region. The main technologies to convert biogas energy into electricity are gas turbines and micro-turbines.

It should be noted that according to Silva et al. (2005), biogas is typically stored at low pressure and can be used in heat generation equipment, such as stoves, furnaces, thermal radiation heaters, etc. A substantial part of the equipment using biogas is still empirically adapted, but with the increase in the number of biodigesters, driven by projects related to the clean development mechanism (CDM), a rapid evolution in the quality of the burners and engines offered on the market is expected.

One should consider that the increasing technological sophistication and industrial activity in modern society has become possible thanks to the various forms of energy available. The dependence of the agricultural sector on fossil fuels is a question that deserves to be discussed. Fuel consumption from this source represents approximately 60.5% of the energy consumed in the

agricultural sector. Their high price, future shortage and the environmental contamination caused by their use, are issues of economic and environmental importance.

Another aspect to be considered is the wasted energy when agro-industrial waste is not harnessed, in addition to the environmental pollution caused by its uncontrolled disposal in these environment. One cannot fail to mention the opportunities that the responsibilities for reducing greenhouse gas emissions taken up by countries through the signing of the Kyoto Protocol and the Clean Development Mechanism, offer for the growth of developing countries.

Conclusion

The obtained results reveal the viability of using poultry litter for biogas production, since the raw material for its production is available in the entire southwest region of Paraná. The presentation of the three treatments proved to be important, because in the three systems the production of significant quantities of biogas was possible, constituting a potential replacement for non-renewable fuel sources derived from petroleum. The biogas yield estimates from poultry litter revealed that a large energy potential is being left unused in the broiler production chain of the southwest region of Paraná - Brazil. The energy from biogas can make the activity more competitive in the region, thereby compensating for the difficulty in obtaining the main inputs for the activity (corn and soybeans). The harnessing of the poultry litter will result in gains of a: (i) economic nature: the energy generated decreases the cost of production with the possibility of selling the energy to the utility; (ii) environmental nature: the treatment of bird waste through anaerobic biodigestion prevents the contamination of rivers and springs with the toxic substances existing in the poultry litter. Additionally, the use of biogas avoids the emission of methane (CH_4) into the atmosphere, contributing to the mitigation of the greenhouse effect; and (iii) social nature: Resources that were previously intended for the payment of energy consumed in the activity, can now be redirected to other purposes, improving the quality of life of people who are directly connected with the activity and also increasing the circulation of money in the region, stimulating sectors of the economy, such as trade.

The study also showed that the electrical power available from biogas generated from poultry litter across the production chain represents around 117,624,599 kWh of electrical power.

Conflicts of Interest

The authors declare there are no ethical, publishing of financial conflicts of interest regarding the data of this study.

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Full Length Research Paper

Applying of sodium nitroprusside (SNP) on postharvest 'Nam Dok Mai No.4' mango fruits delay ripening and maintain quality

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The aim of this study was to evaluate the concentrations of sodium nitroprusside (SNP), a nitric oxide donor, on postharvest ripening and quality of mango fruit cv. Nam Dok Mai No.4. The fruits were dipped in 1 or 2 mM of SNP solution for 30 min while control fruits were dipped in tap water for 30 min. After treatment, all fruits were stored for 18 days at 13°C and RH 90%. Both SNP doses showed significantly reduced ethylene production and respiration rate, maintained the fruit firmness, decreased the changes in total soluble solids and titratable acidity, and reduced fruit weight loss. The treatments of SNP also delayed color development in fruit peel and pulp. SNP-treated fruits showed higher L* and hue angle values and lower a* and b* values in comparison with control fruits. No significant differences were found between 1 and 2 mM SNP treatments. SNP applying on dose of 1mM to postharvest 'Nam Dok Mai No.4' mango fruits and then stored at 13°C changed ethylene production and respiration rates, maintained firmness pulp, decreased weight loss and slowed the changes in fruit peel and pulp color, total soluble solids (TSS) and titratable acidity (TA),.

Key words: *Mangifera indica* L., sodium nitroprusside, biochemical changes, fruit quality.

INTRODUCTION

Nitric oxide (NO) is recognized as a biological messenger in plants. It is a highly reactive gaseous free radical and is soluble in water and lipid (Hayat et al., 2010). NO can mediate various pathophysiological and developmental processes, including the expression of defence-related genes, programmed cell death, stomatal closure, seed generation and root development (Neill et al., 2003). Optimum NO levels could delay the climacteric phase of many tropical fruits and prolong the post-harvest shelf life of a wide range of horticultural crops by preventing ripening and senescence (Singh et al., 2013). NO-fumigated fruits reduced ethylene production due to binding of NO with 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC oxidase to form a stable ternary complex, thus limiting ethylene production (Tierney et al., 2005). Sodium nitroprusside (SNP – a NO donor) solution

at 1 mM was able to inhibit pectin solubilization/depolymerization, delay the increase in total phenolic content, and lower DPPH radical scavenging activity in plum fruits applied at postharvest during 120 days at 2°C (Li et al., 2007). Peach fruits treated with 1 mM SNP prolonged shelf life by reducing ethylene production rate, increasing firmness and antioxidant activity potential of the enzymes such as superoxide dismutase, and catalases (Sis et al., 2012).

Thailand is one of the most important countries in Asian continent in exporting mangos and the cv. Nam Dok Mai is a well known cultivar, with a fibreless, delicious taste and sweet aroma. However, as a fleshy fruit, the losses resulting in senescence, desiccation, physiological disorders, mechanical injuries and microbial spoilages can occur at any point from harvest to utilization (Wu, 2010). Up to 30% of mango products are lost due to improper post-harvest operations (Malik et al., 2005). Mango storage time is limited to 2 to 3 weeks in normal air at 10 to 15°C depending on the cultivar (Yahia, 1999). Application of 1-MCP has potential for the commercial control of ripening and softening of harvested mangos. However, the use of appropriate 1-MCP concentration still requires being investigated further (Sivakumar et al., 2011). The treatment of NO on mango fruits was mostly reported in gas (fumigation with gaseous NO). In practice, the gaseous NO treatment requires postharvest infrastructures, therefore the dipping with SNP solution will be more suitable than gaseous treatment (Sis et al., 2012). The SNP reaction releases NO and cyanide (CN) which is known as a toxic chemical agent. Free CN radical may bind and inactivate tissue cytochrome oxidase and prevent oxidative phosphorylation (Friederich and Butterworth, 1995). Minimum absorbed lethal dose (LD_{50}) of CN in humans was estimated to be 1.4 to 3.0 mg/kg (Way, 1981). Therefore, using SNP in postharvest management should be cautious for human safety in term of the concentration and also ventilation during treatment. Up to this time, data on applying of NO on Thai mangos are still limited, and in particular no information has been reported on SNP treatment on 'Nam Dok Mai' mangos. The aim of this study was to determine different concentrations of SNP on the effects of physiological and biochemical properties of 'Nam Dok Mai No.4' mango fruits during cold storage.

MATERIALS AND METHODS

'Nam Dok Mai No.4' mango fruits at the mature stage were purchased from the exporting company in Bangkok, Thailand. The fruit were selected for uniform, size and color, besides of disease and mechanical damage. SNP (HIMEDIA company, India - molecular weight: 297.95 g mol^{-1}) was dissolved in distilled water before using. In the preliminary study, we found that 0.5 mM SNP did not have clear effect on mango quality during storage, and 4 mM SNP caused the damage to mango fruit skin). In this research, the fruits were dipped in 1 or 2 mM of SNP solution for 30 min at the ambient temperature. Control samples were dipped in water

following the same procedure as fruits treated in SNP. Each sample fruit were composed by 3 fruits x 3 replications. After treatment all fruits were stored at refrigerated chambers at 13°C for 18 days with RH 90%.

Measurements of ethylene production and respiration rate

Fruits were put in a closed plastic box, and then incubated for 1 hour at the storage condition. A 1 mL gas sample from each plastic box was injected into the gas chromatograph (Shimadzu GC-8A and Shimadzu GC-14B, Japan) to measure CO_2 and C_2H_4 concentrations. The fruit respiration rate was expressed as mg CO_2 kg^{-1} h^{-1} and ethylene production was expressed as µL C_2H_4 kg^{-1} h^{-1}.

Measurements of quality

Fruit firmness was measured using the texture analyser (TA.XT plus, UK) and the results were expressed in Newtons (N). The color changes in the fruit peel and pulp were measured using a colorimeter (Konica Minolta CR-400, Japan). The color was expressed as the values of L*, a*, b* and hue angle (h). The values of L* describes the lightness increasing from black to white. The values of a* and b* describe the color position. Negative a* values indicate green while positive values indicate magenta. Negative b* values indicate blue and positive values indicate yellow. h describes the hue angle. It ranges from 0 to 360° (h=0°: red; h=90°: yellow; h =180°: green; h=270°: blue). Fruit weight losses were expressed as a percentage of weight loss relative to the initial weight. Total soluble solids (TSS) were measured using the digital refractometer (Atago, PAL-1, Japan) and the results expressed as °Brix. To measure titratable acidity (TA), fruit juice was titrated against 0.1 N NaOH until pH 8.1, and the results were expressed as % of citric acid.

Statistical analysis

All statistical analyses were performed with SPSS software ®. The experiment was carried out in a completely randomized design with three replicates for each treatment. All parameters were measured at day 0 and then at three-day intervals until 18 days. The data were expressed as the mean±SE. Differences between SNP-treated and the control fruits of the same day were compared using Fisher's least significant differences (LSD) F-test with p<(0.05).

RESULTS

Respiration rate and ethylene production

Respiration rate in all fruits increased from day 0 and reached the respiration peaks at day 12 then decreased. Both SNP treatments significantly decreased respiration rates at days 3, 6 and 12. The rate of respiration in untreated fruits was 1.5-fold higher than in treated fruits (Figure 1a). The SNP treatments significantly inhibited ethylene production during cold storage. Ethylene production in the control treatment was 2-fold higher than in the SNP treatment at day 3 and kept that rate until the last day of storage (Figure 1b). The treatment of 1 mM SNP was more effective than 2 mM SNP in the inhibition
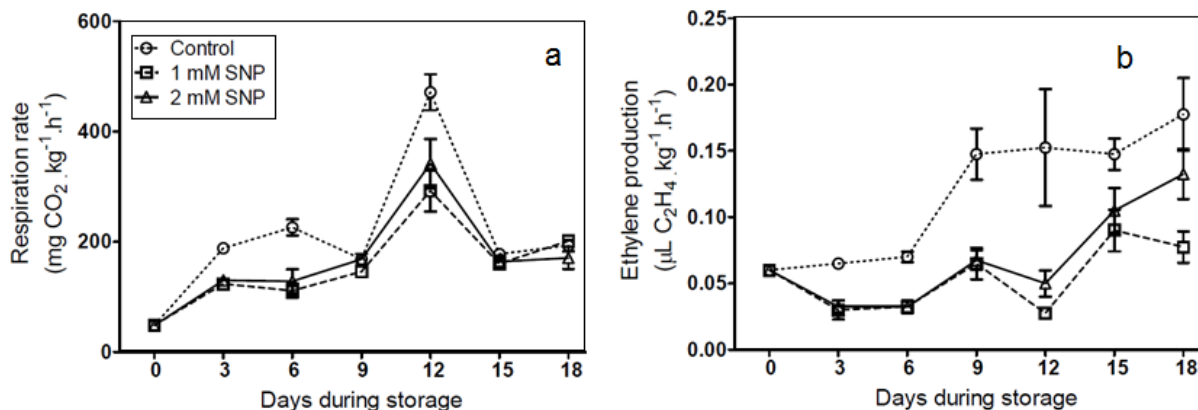
Figure 1. Effect of SNP treatment on respiration rate (a) and ethylene production (b) of 'Nam Dok Mai No.4' mango fruit during cold storage at 13°C for 18 days. Data represent the means ± SE, n = 3. Vertical bars represent SE of the means and are not visible when the values are smaller than the symbols.

of respiration rate and ethylene production in mango fruits during cold storage.

Firmness, weight loss, TSS and TA

The firmness in the control fruits reduced slightly from day 3 to day 12 then decreased sharply to day 18, while the treated fruits exhibited the maintenance of fruit firmness. Fruit treated with 1 or 2 mM SNP was significantly firmer than untreated fruit during cold storage except day 6 or days 6 and 12, respectively (Figure 2a). The SNP treated fruits exhibited significantly lower weight loss than untreated fruits during cold storage. Significant decrease in weight loss in 1 mM SNP was observed throughout the storage period, while 2 mM SNP showed significant differences in weight loss only at later periods (days 12, 15 and 18) (Figure 2b). Fruit treated with SNP exhibited lower values in TSS and higher values in TA during cold storage. The significant differences between treated and untreated fruits in TSS were found on days 6 and 18 and in TA on days 9 and 15, respectively (Figures 2c and d). At 1 mM SNP was more effective than 2 mM SNP in decreasing the changes in TSS and TA with no significant difference.

Fruit color

Treatment with SNP inhibited the changes in mango peel and pulp color during storage, especially at the later periods. Significantly lower a* and b* values in the fruit peel were measured at days 9, 12, 15, 18 and days 9 and 12, respectively. The treatment of 1 mM SNP seemed to be more effective than 2 mM SNP in decreasing the changes in the hue angle of fruit peel. Fruits treated with 1 mM SNP significantly decreased the change in hue angle from days 12 to 18 while 2 mM SNP had only one

period of significant difference (day 18) compared with untreated fruits. Significant differences in fruit pulp color were found at days 15 and 18, with higher values in L* and hue angle and lower values in b* in SNP treated fruits compared with the control fruits (Table 1).

DISCUSSION

Mangos are climacteric fruits and their ripening is characterized by the burst of ethylene production and a respiration climacteric followed by a series of biochemical changes (Reddy and Srivastava, 1999). In our studies, SNP treatments inhibited ethylene production in mango fruits during cold storage. The inhibition of ethylene may suppress the ripening process including maintaining the firmness pulp, suppressing the increase in TSS (on day 6 and day 8), suppressing the decrease in TA (on day 9 and day 15). In addition, the fruits treated with 1 mM SNP exhibited the significantly lower weight loss during storage. During ripening process of mango fruits, the peel color changes from green to yellow, and the pulp color changes from white to yellow. These changes are associated with the changes in the values of L*, a*, b*, and hue angle. In this research, SNP treatments decreased the color development during mango ripening due to inhibiting the increase in a* and b*, and the decrease in h angle in fruit peel, and inhibiting the increase in b* values and the decrease in L* values and h angle in fruit pulp. These effects resulted in delaying mango fruit ripening.

The effects of NO on postharvest fruits were pronounced in some papers. NO fumigation at 60 µL.L⁻¹ effectively suppressed ethylene formation and respiratory rate, reduced weight loss, maintained firmness and delayed changes in peel color and TSS in papaya fruits during 20 days of storage (Li et al., 2014). Post-harvest exposure of 'Amber Jewel' plums to NO gas with 10
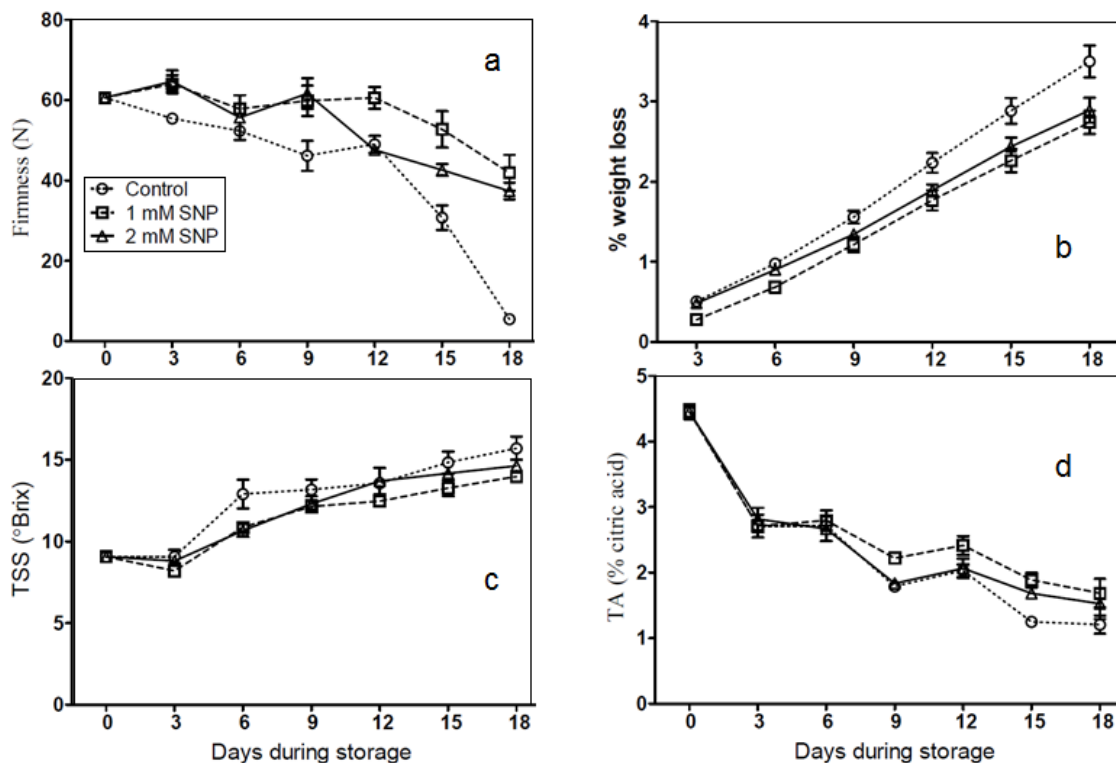
Figure 2. Effect of SNP concentrations on the changes of firmness (a), weight loss (b), TSS (c) and TA (d) of 'Nam Dok Mai No.4' mango fruit during cold storage at 13°C for 18 days. Data represent the means ± SE, n = 3. Vertical bars represent SE of the means.

Table 1. Effect of SNP treatment on the color changes of 'Nam Dok Mai No.4' mango fruits during storage at 13°C.

Peel colour	Treatment (mM)	Storage time (days)						
		0	3	6	9	12	15	18
L*	Control	74.5	70.7 ± 0.5	71.0 ± 2.3	71.6 ± 2.2	73.9 ± 0.3	75.4 ± 0.5	73.0 ± 1.0
	1	74.5	70.3 ± 0.2	72.3 ± 0.9	70.7 ± 1.2	73.2 ± 0.3	74.1 ± 0.6	72.4 ± 1.3
	2	74.5	71.8 ± 0.7	68.9 ± 1.0	69.9 ± 0.7	73.3 ± 0.5	74.2 ± 2.1	72.1 ± 0.7
a*	Control	-5.2	-7.3 ± 0.4	-6.8 ± 0.6	-5.2 ± 0.2[a]	-3.8 ± 0.3[a]	-2.1 ± 0.4[a]	-0.6 ± 0.4[a]
	1	-5.2	-7.8 ± 0.7	-7.4 ± 0.5	-6.4 ± 0.3[b]	-5.5 ± 0.2[b]	-3.4 ± 0.1[b]	-2.3 ± 0.2[b]
	2	-5.2	-6.7 ± 1.3	-8.3 ± 0.1	-6.2 ± 0.1[b]	-5.5 ± 0.6[b]	-3.5 ± 0.2[b]	-2.8 ± 0.1[b]
b*	Control	25.8	24.2 ± 0.2	27.0 ± 1.9	25.6 ± 1.1[a]	27.0 ± 0.6[a]	25.8 ± 0.5	31.1 ± 0.5
	1	25.8	23.8 ± 0.6	26.1 ± 0.3	22.9 ± 0.5[b]	24.7 ± 0.4[b]	25.2 ± 0.4	30.7 ± 1.0
	2	25.8	23.6 ± 0.1	26.7 ± 0.9	23.4 ± 0.2[ab]	24.7 ± 0.4[b]	25.4 ± 0.7	29.9 ± 1.1
Hue angle (h)	Control	101.2	106.8± 0.6	101.9 ± 4.4	96.9 ± 4.1	95.1 ± 3.9[b]	92.2 ± 3.3[b]	93.6 ± 2.3[b]
	1	101.2	108.1± 1.3	105.8 ± 1.2	102.4 ± 3.6	103.1± 1.0[a]	101.4± 1.3[a]	99.7 ± 1.2[a]
	2	101.2	105.5± 2.9	107.6 ± 0.8	104.2 ± 0.9	101.0± 0.8[ab]	94.6 ± 3.2[ab]	100.0± 0.6[a]
Pulp colour								
L*	Control	89.5	86.5 ± 0.1	86.6 ± 0.8	85.7 ± 0.9	84.7 ± 0.3	83.5 ± 0.3[b]	82.2 ± 0.3[b]
	1	89.5	86.2 ± 0.1	86.2 ± 0.4	86.9 ± 0.3	86.5 ± 0.7	85.6 ± 0.2[a]	84.7 ± 0.5[a]
	2	89.5	86.7 ± 0.3	86.7 ± 0.4	86.0 ± 0.2	85.4 ± 0.6	84.5 ± 0.4[ab]	84.4 ± 0.7[a]
a*	Control	0.6	-0.4 ± 0.5	-1.3 ± 0.2	-0.6 ± 0.1	0.2 ± 0.1	1.0 ± 0.2	1.3 ± 0.4
	1	0.6	-1.1 ± 0.2	-1.1 ± 0.2	-0.6 ± 0.5	-0.4 ± 0.4	0.8 ± 0.2	0.6 ± 0.2
	2	0.6	-0.6 ± 0.3	-1.6 ± 0.2	-0.9 ± 0.3	-0.4 ± 0.2	0.7 ± 0.1	0.8 ± 0.0
b*	Control	9.3	8.3 ± 0.2	10.3 ± 1.4	12.6 ± 1.2	18.4 ± 1.2	24.0 ± 0.5[a]	30.6 ± 0.8
	1	9.3	9.2 ± 0.8	10.7 ± 1.5	11.0 ± 0.8	16.7 ± 1.6	19.3 ± 0.8[b]	25.1 ± 1.7

Table 1. Contd.

	2	9.3	9.7 ± 1.0	11.0 ± 1.4	11.1 ± 0.8	16.7 ± 0.8	22.0 ± 2.0[a]	25.8 ± 2.2
Hue angle (H)	Control	91.1	90.5 ± 0.5	92.7 ± 0.2	90.0 ± 0.4[b]	90.6 ± 0.3	89.0 ± 0.4[b]	88.3 ± 0.8[b]
(h)	1	91.1	91.4 ± 1.2	92.6 ± 0.3	91.3 ± 0.4[ab]	90.8 ± 1.4	91.5 ± 0.5[a]	91.9 ± 0.6[a]
	2	91.1	89.9 ± 0.2	94.1 ± 0.9	91.6 ± 0.6[a]	91.5 ± 0.4	92.1 ± 0.5[a]	91.2 ± 1.0[a]

Data represent the means ± SE, n = 3. Values followed by the different letters within the same column indicate significant differences among treatments at $p ≤ 0.05$ (LSD test).

Figure 3. The peel and pulp color in mango fruits treated with SNP at 1 and 2 mM and control fruits on day 18 of storage at 13°C.

µL.L⁻¹ delayed ripening by 3 to 4 days at 21±1°C by suppressing respiration and ethylene production, delaying the color development, maintaining the firmness and decreasing changes in TA in plum fruits (Singh et al., 2009).

NO fumigation was reported to reduce respiration in 'Kensington Pride' mango fruits during ripening at 21°C (Zaharah and Singh, 2011). Similarly, NO treatment was found to reduce respiration in plums (Singh et al., 2009), peaches (Flores et al., 2008) and strawberries (Zhu and Zhou, 2007). Our results also showed that SNP treatment at 1 mM inhibited the respiration rate and weight loss during storage. The maintenance of fruit weight may be due to a suppression of respiration rate in the SNP treated fruit. NO affects the mitochondrial activity in plant cells and reduces total cell respiration due to its inhibitory effect on the cytochrome leading to decreasing respiration (Hayat et al., 2010).

The delay of the ripening and the maintenance of fruit quality in SNP treated fruits may be explained by suppressing biochemical changes in ethylene or respiration pathway corroborating that SNP can regulate ethylene dependent or independent pathway by activity enzymatic or genes expression (Cheng et al., 2009). In our research, the treatment with 1 mM SNP was more effective than 2 mM SNP in inhibiting ethylene production and maintaining the quality of 'Nam Dok Mai' mango fruits during storage at 13°C (Figure 3). The exogenous

application of NO markedly delayed maturation and senescence in fruits where NO effect was stoichiometrically related to ethylene suppression (Leshem and Wills, 1998). It is known that NO and ethylene are both gaseous hormones in plants, which act antagonistically (Manjunatha et al., 2010). The decrease of ethylene production in bananas was associated with the reduction in the activity of ACO and the expression of the MA-ACO1 gene (Cheng et al., 2009). In tomato fruits, NO application reduced the transcription of ACO genes such as *LeACO1*, *LeACOH2*, and *LeACO4* and the level of ethylene emission were reduced (Eum et al., 2009).

The inhibition of ethylene biosynthesis by NO was also reported in many fruits such as: strawberries (Zhu and Zhou, 2007), peaches (Zhu et al., 2006), plums (Singh et al., 2009) and papayas (Li et al., 2014). Similarly, peach fruits treated with 1 mM SNP then stored at 1°C effectively prevented the development of autocatalytic ethylene biosynthesis (Sis et al., 2012).

Conclusions

SNP applying on dose of 1 mM to postharvest 'Nam Dok Mai No.4' mango fruits and then stored at 13°C changed ethylene production and respiration rates, maintained firmness pulp, decreased weight loss and slowed the changes in fruit peel and pulp color, TSS and TA.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Physiological responses of *Medicago truncatula* growth under prolonged hypoxia stress

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Flooding is an important environmental factor restricting plant growth and productivity throughout the world. Growth, organic solute accumulation and proteolytic activity of *Medicago truncatula* were determined in hydroponically grown plants exposed to hypoxia during 30, 45 and 60 days. Obtained results showed that hypoxia until 45 days have no significant impact on the dry matter production. However, after 60 days of treatment, a decrease in the shoot and root growth was observed. In addition, a development of adventitious roots is shown in the hypocotyle of treated plant. In fact, the water content varied in response to the hypoxia. The soluble carbohydrates content was still stable on the shoot of treated plant until 45 day. Whereas, it was registered that the content of non-reducing soluble sugar present almost all of total soluble sugars, and that the accumulation of this sugars fraction in more important in the roots of hypoxia plants in comparison to the aerial organs. The proline content measured in stressed plants was increased with time over control plants. Contrary, hypoxia decreased the total endopeptidase activity in *M. truncatula* roots. It could be concluded that *M. truncatula* can be support the prolonged hypoxic stress by developing adventitious roots and accumulating proline.

Key words: *Medicago truncatula*, hypoxia, adventitious roots, soluble sugars, proline, endopeptidase activity.

INTRODUCTION

The lack or the excess of the water in the rhizosphere present a critical factor on plant growth. Indeed, drought or waterlogging affect plant characteristics (Iqbal et al., 2009; Ibraheem et al., 2011; Promkhambut et al., 2011). Flooding is one of the most significant abiotic stresses, it causes a slow transfer of dissolved oxygen in the waterfilled pore space of the soil, and plants can have to face to the lack of oxygen during all stage of its growth. Seed imbibitions, flood irrigation, floods and excess of rainfall are examples of natural conditions leading to root

hypoxia or anoxia. These conditions can severely disturb plants development (Voesenek and Bailey-Serres, 2013). The oxygen deficiency is thought to be a major determinant in the adverse effects of flooding on crops and other plant species (Mommer et al., 2004). Not all plant species can survive to the waterlogging conditions, only some species are able to adapt to this stress by developing some physiological and morphological mechanisms like adventitious roots and aerenchyma (Vartapetian and Jackson, 1997; Vignolio et al., 1999).

Nevertheless, this adaptive capacity depends on the plant species (Poot and Lambers, 2003). Among species, legumes represent the third largest group of angiosperms and the second largest group of food and feed crops grown globally. They include 670 to 750 genera and 18000 to 19000 species. It regroups varied food and feed crop species, such as clover, chickpea, soybean, pea, alfalfa and mungbean. Grain legumes provide about one-third of all dietary protein nitrogen and one-third of processed vegetable oil for human consumption (Graham and Vance, 2003). Indeed, some varieties such as soybean and *Pongamia pinnata* takes more attention because of their high seed oil content how can be used like a biofuel (Scott et al., 2008). However, Leguminosae are able to evolve a symbiotic relationship with specific soil bacteria, called Rhizobia (de Faria et al., 1989). As a result, many legumes are sensitive to waterlogging, showing reductions in root growth, nodulation process, nitrogen fixation and photosynthesis activity (Linkemer et al., 1998; Bacanamwo and Parcell, 1999). To develop more knowledge on legumes, *Medicago truncatula* get more attention because of its short growth cycle and its small genome (500 to 550 Mpb) (Young et al., 2005). It is a model plant and presents the subject of numerous researches that studying the symbiotic and the mycorrhizal symbiosis (Ané et al., 2004), the abiotic stress tolerance (Narasimhamoorthy et al., 2007), the disease resistance (Ameline-Torregrosa et al., 2008). In this work, we tried to investigate the impact of the prolonged time of hypoxia on the growth of *M. truncatula*. We follow the production of dry matter, the water content, the sugar content, the shoot proline, and the total endopeptidase activity after 30, 45 and 60 days of hypoxia.

MATERIALS AND METHODS

Biological material and growth conditions

Seeds of *M. truncatula* A17 were sterilized with sodium hypochlorite (0.6%), rinsed with distilled water and imbibed during 2 h. Seeds are placed on moist filter paper in a Petri dish and incubated at 25°C. After 5 days, the seedlings were grown hydroponically into glass container. The nutrient solution contained macronutrients: 2.25 mM KNO_3; 0.25 mM Ca $(NO_3)_2$; 0.35 mM KH_2PO_4 and 0.075 mM $MgSO_4$, micronutrients: 268.6 µM EDTA-Fe; 8.9 µM $MnSO_4$; 24.1 µM H_3BO_3; 1.7 µM $ZnSO_4$ and 3.9 µM $CuSO_4$ (Saglio and Pradet, 1980).

Hypoxic treatment was applied at the second leaf stage by stopping air bubbling, whereas control plants were continuously aerated. Seedlings were grown in a growth room at 25°C/20°C (day/night) with 18 h/6 h (day/night) photoperiod and 75% relative humidity.

Vegetative growth analysis

Growth parameters were evaluated after 30, 45 and 60 days of hypoxia treatment. Plants were harvested, divided into roots and shoots. Roots were washed in distilled water. Fresh weights (FW) were immediately determined for roots and shoots. Dry weights (DW) were obtained by weighing the plant material after drying at 80°C until a constant mass was reached. Water content (WC) was calculated as (FW-DW) / DW.

Soluble carbohydrates and proline determination

Soluble carbohydrates, total and reduced, were determined as described by Aschi -Smiti et al. (2003). Free proline was quantified spectrophotometrically by the ninhydrin method according to Bates et al. (1973). The plant material was homogenized in 3% (w/v) aqueous sulfosalicylic acid and the homogenate was centrifuged at 14000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of 2 ml of ninhydrin acid and 2 ml of glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and absorbance was read at 520 nm.

Proteolytic activity

Proteolytic activity in shoot crude extracts was determined spectrophotometrically by following the digestion of azocasein at 440 nm (Brouquisse et al., 1998).

Statistical analysis

Three biological replications were used in this study. Statistical data analysis was made using Student's t-test. The results are given as means with standard errors of at least 10 samples per treatment. The significance of differences between the control and the treatment mean values was determined at the significance level of $p < 0.05$.

RESULTS

Vegetative growth analysis

In the present study, hypoxia was applied at the second leaf stage of *M. truncatula* growth. In function of time, the biomass production of *M. truncatula* was increased and the differences between control and treated plant were not significant up to 45 days of treatment. At 60 days of hypoxia decreased the growth of shoot and root respectively by 44 and 52% in comparison to control plants (Figure 1).

In fact, hypoxia decreased the water content in shoot respectively by 14, 36 and 33% after 30, 45 and 60 days of treatment (Figure 2A). While, the water content in roots was decreased after 45 and 60 days of hypoxia and an augmentation of the water content was noticed after 30 days estimated by 34% (Figure 2B).

Nevertheless, prolonged root hypoxia (60 days) decreased the development of the whole plant. The picture presents the development of adventitious roots on the hypocotyle of *M. truncatula.* In the present study, compared with the control plant that had no adventitious root, it was observed the petrifaction of the initial root system (Figure 3).
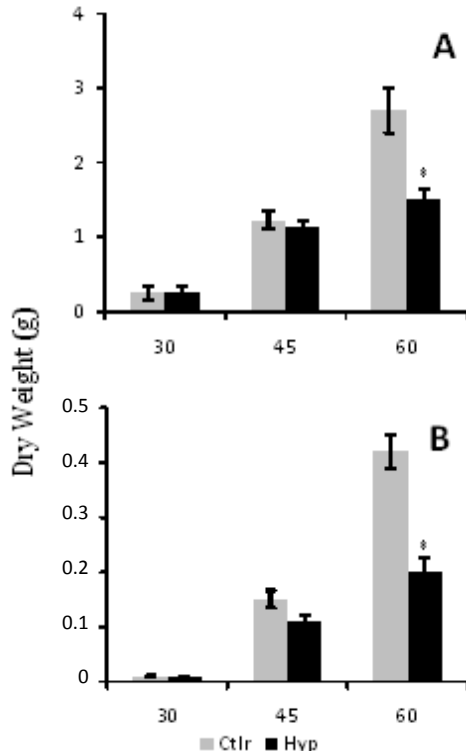
Figure 1. Shoot (A) and root (B) dry weight (DW) of control (grey) and hypoxia treatment (black) of *M. truncatula* during 30, 45 and 60 days. Values are the mean ± S.E. from ten measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$ in comparison to the control.
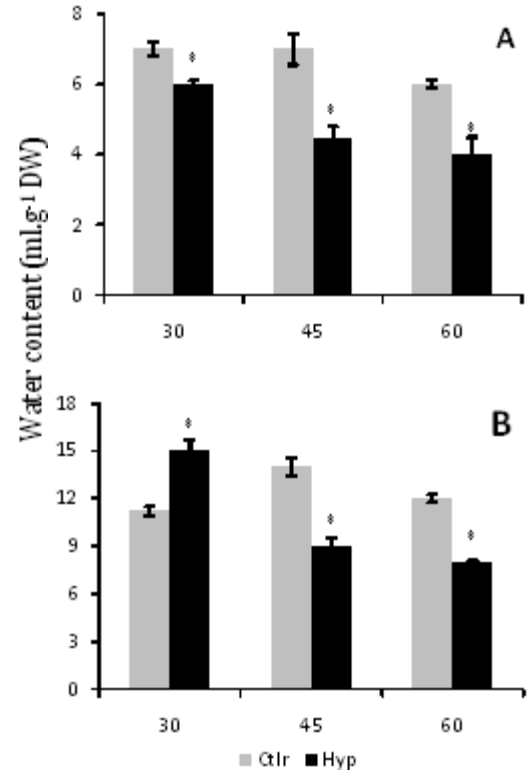
Figure 2. Shoot (A) and root (B) water content of control (grey) and hypoxia treatment (black) of *M. truncatula* for 30, 45 and 60 days. Values are the mean ± S.E. from ten measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$ in comparison to the control.

Soluble carbohydrates

Data presented in Figure 4 revealed that the content of total soluble sugars (TSS) in shoots still stable up to 45 days of treatment. After 60 days of hypoxic treatment, an augmentation of TSS was observed (Figure $4A_1$). For the underground organ, the sugar content was increased by 50% between 30 and 45 days in control plants, while it decreased at 60 days by 55%. On the other hand, in treated plants, hypoxia increased total root sugar (Figure $4A_2$).

The results of two fractions of soluble sugar: reducing (RS) and non-reducing sugar (NRS) showed that the content on NRS (Figure 4C) is more important in *M. truncatula* organs (Figure 4B). In fact, deficient plant on oxygen presents an elevated content on NRS in their shoots, whereas it was decreased in the root with the treatment. However, at 45 and 60 days the NRS was decreased by 21 and 95% in comparison to the content at 30 days. In parallel to this decreases, it showed an augmentation of RS on stressed roots by 75 and 55% at 30 days.

Proline and endopeptidase activity

Proline is uncharged amino acid, it characterize by a neutral pH and a highly water solubility. The results presented in Figure 5A revealed that the proline content of control plant shoots is stable up to 45 days, at 60 days; we showed an augmentation by 40%. However, hypoxia induced synthesise of proline on function of time. The increase estimated by 25 and 30% respectively at 45 and 60 days in comparison to control plant.

The variation of the total endopeptidase activity (Figure 5B) in roots of control plant was increased by 37 and 45% at 45 and 60 days, in comparison to the content obtained at 30 days. On contrary, hypoxia decreased the endopeptidase activity in roots. This reduction estimated by 39, 48 and 55% at 30, 45 and 60 days in comparison to control.

DISCUSSION

Waterlogging stress is known to cause a number of morphological and anatomical changes in plants (Sairam
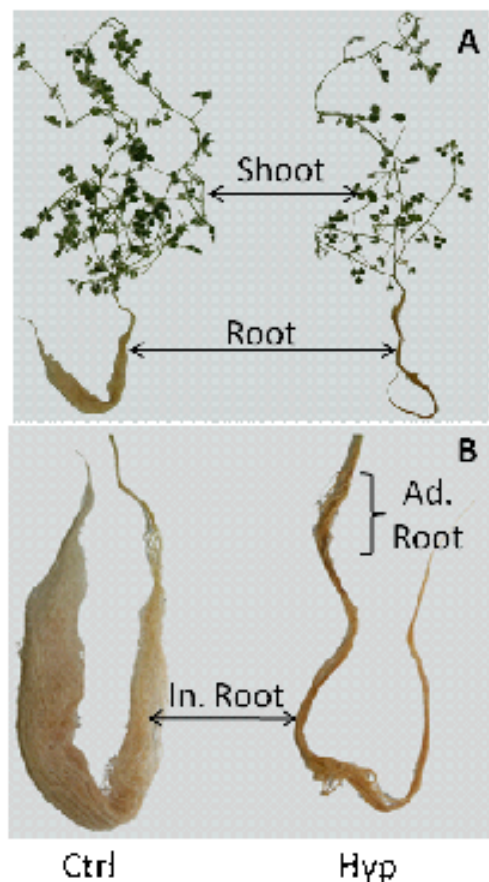
Figure 3. Morphological aspect of *M. truncatula* (A) and roots (B) after 60 days of hypoxia treatment. Ad: Adventitious roots; In: initial.

et al., 2008). For example, the apparition of hypertrophied lenticels on roots, the formation of adventitious roots (Malik et al., 2001), the development of aerenchyma (Evans, 2004; Abiko et al., 2012) were observed under flooding stress. In this work, the application of prolonged hypoxia treatment to *M. truncatula* caused the apparition of adventitious roots on the hypocotyle. In spite this adaptation to the hypoxia; it was noticed a diminution of the plant water content. This decrease in the water status can be the result of the reduction of the stomata conductance. This phenomenon is considered one of the first plant responses to waterlogging (Folzer et al., 2006). Indeed, plants exposed to flooding stress exhibit increased stomata resistance as well as, limited water uptake leading to internal water deficit (Parent et al., 2008). Moreover the lack of oxygen can reduce hydraulic conductivity due to hampered root permeability (Else et al., 2001). Furthermore, *M. truncatula* present decline of its hall growth. This decrease can be the result of the petrifaction on the principal root that can caused perturbation of the element uptake's (Horchani et al., 2010). In plus, the oxygen deficiency in the growth medium can cause a decline of net photosynthetic rate

(Aloui et al., 2011).

Another adaptation road of plant to survive under hypoxia is the shifting of the energy metabolism from aerobic to anaerobic mode since the energy requirements of the tissue is greatly restricted as very few ATPs are generated per molecule of glucose (Jackson and Drew, 1984). Thus, maintaining adequate levels of readily fermentable sugars under oxygen deficient in roots is one of the adaptive mechanisms to hypoxia (Xia and Saglio, 1992; Sairam et al., 2009). Moreover, waterlogging, in tolerant genotypes, induces to increase the content of reducing sugar through increased activity of sucrose synthase by increasing the level expression of its mRNA (Sairam et al., 2009). Therefore, the availability of sufficient sugar reserves in the roots with the increased activity of TSS to provide reducing sugars for anaerobic respiration is one of the important mechanisms of waterlogging tolerance. In our results, *M. truncatula* present more non-reducing sugar than reducing form. Therefore, sucrose represents almost all of the total soluble sugars in this legume. The concentration of soluble carbohydrate in *M. truncatula* was increased during the prolonged long-term oxygen deficit, and we register an augmentation of the root and shoot content on reducing sugar on function of time (Kumutha et al., 2008). In addition the maintenance of elevated content of shoots non-reducing sugar on parallel to its spectacular diminution in root. These results are in agreement with those obtained by Germain and collaborators (1997) on tomato. Moreover, maintaining of elevated quantity of non-reducing sugar in root until 45 days car suggesting the eventual transport of sucrose from shoot to root (Yu et al., 2012).

Concentrations of free proline in the leaves of *M. truncatula* increased synchronously with time of hypoxia treatment. Proline accumulation is known to have multifunctional role on the defense mechanisms developed by plants grown under various stress (Nanjo et al., 2003). It's related also to the non-enzymatic detoxification of free radicals (superoxide, peroxide or hydroxyl) that are generated excessively under stress (Radyukina et al., 2008). It plays an important role of osmolyte for osmotic adjustment and contributes to the stabilization of cell structures, protection of membranes and proteins against reactive oxygen species (Steffens et al., 2012). The authors explain such ability of this amino acid by the presence of tertiary carbon which can form stable radical tearing off free radical reactions induced by ROS (Radyukina et al., 2008). In roots of *M. truncatula* the proteolytic activity was increased with time but still fewer that obtained in roots of control plant. In fact, the increase of the proteolytic activity is explained by the nature and the development of tissues. Indeed, roots of control plants are much vacuolated and the endopeptidase activity quantified with the azocasein characterizes vacuole. These results are similar to those found by Aschi-Smiti and collaborators (2003) on *Trifolium subterraneum.*
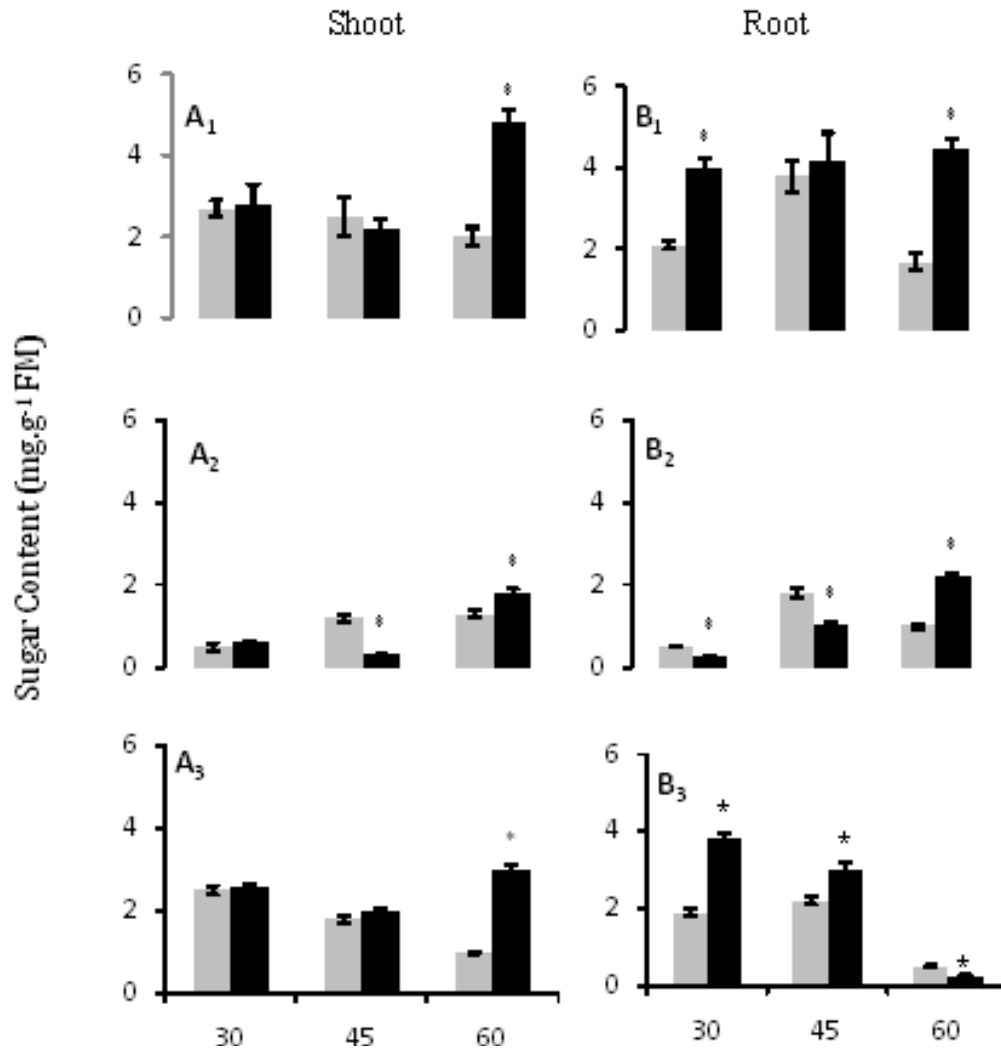
Figure 4. Shoot (A) and root (B) sugar content of control (grey) and hypoxia treatment (black) of *M. truncatula* after 30, 45 and 60 days. A_1 and A_2: Total sugar; B_1 and B_2: Reducing sugar and A_3 and B_3: Non-reducing sugar. Values are the mean ± S.E. from ten measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$ in comparison to the control.

The lowest proteolytic activity was found in hypoxic's root can be the result of the purification and the necrosis of root under hypoxia. Since that adventitious roots ensure survive and nutrition of *M. truncatula*; this roots are physiologically young and haven't important vacuoles. These results are consistent with those obtained on maize under dark stress by Brouquisse et al. (1998) and on *Trifolium subterraneum* grown in hypoxia by Aschi-Smiti et al. (2003).

Conclusion

*M. truncatula* seem supporting the prolonged hypoxia stress. Its capacity to develop adventitious roots, accumulating proline and getting an elevated content of sugar help it to achieve its biological cycle. Indeed, it is important to study the molecular and the genetic basis of this adaptation to oxygen deficiency of this model plant.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS
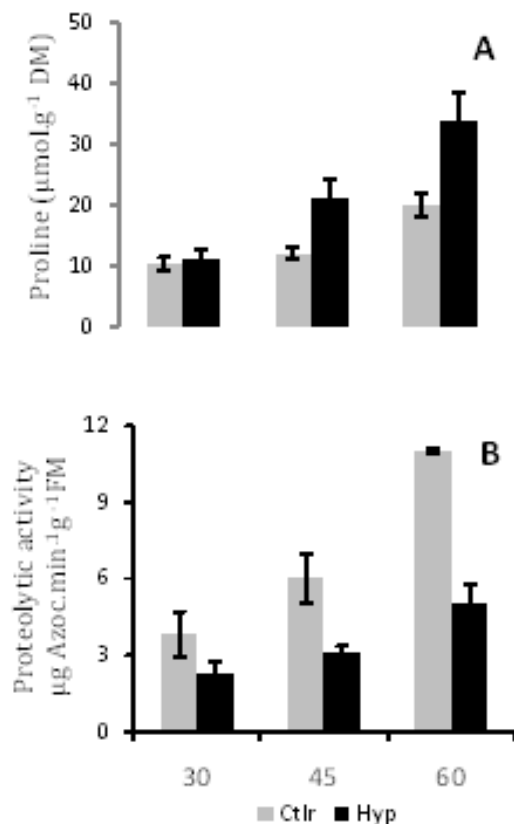
Figure 5. Shoot's proline (A) and root's proteolytic activity (B) of control (grey) and hypoxia treatment (black) of *M. truncatula* after 30, 45 and 60 days. Values are the mean ± S.E. from ten measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of p<0.05 in comparison to the control.

numerous harvests.

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Full Length Research Paper

Stability and genetic divergence study of single cross hybrids in maize (*Zea mays* L.)

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A study of stability and genetic divergence was carried out on 22 experimental maize hybrids for 3 years, at the Maize Breeding Research Station, Poonch, India, to evaluate potential crosses particularly for double cross hybrid with a view that well developed single cross hybrids represent an interesting potential base population for double cross hybrid, as they have high and stable yield and desirable agronomic characteristics, with large proportions of favourable loci previously selected. Considering the estimation of stability and genetic diversity together in the present investigation, both directional crosses based on most diverse and stable parents namely PHM 10 x PHM 23, PHM 10 x 35 and PHM 10 x PHM 31 may be recommended which can throw out superior segregants in hybridization.

Key words: Maize hybrid, stability, genetic divergence.

INTRODUCTION

Stability of the genetic divergence genotypes realized in hybrids may be potential parents for greater frequency of partially dominant alleles (Koutsika and Karagounis, 2005). However, the changing environmental conditions affect the performance of maize genotypes which requires a breeding programme that needs to take into account the consequences of environment and genotype interaction in the selection and release of improved varieties. Allard and Bradshaw (1964) suggested that the selection of genotypes should be based on least interactions with environments. Accordingly, well developed single cross hybrids represent an interesting potential base population for double cross hybrid, as they have been previously tested in various environments. Thus, these hybrids generally present high and stable yield and desirable agronomic characteristics, with large proportions

of favourable loci previously selected (Amorim and Souza, 2005).

The study of genetic divergence can assist in the choice of genotypes to be used in breeding programs for the development of new populations (Cruz and Regazzi, 1997). Genetic divergence is related to the degree of distance between populations in the set of genetic characters that differ between the populations. The more genetically diverse parents, the greater the chances of obtaining higher heterotic expression in F1s and broad spectrum of variability in segregating population (Shekhawat et al., 2001). However, in most cases genetic distance is positively correlated with heterosis (Paterniani et al., 2008). Thus, the magnitude of heterosis is generally proportional to the genetic distance between the parents. According to Cargnelutti et al. (2008), genetic

Table 1. Pooled analysis of variance over environments for different traits in maize hybrids

Source of variation	df	Grain Yield kg./ha.	Days to Tasselling	Days to Maturity	Plant Height (cm)	Shelling %
Varieties	21.00	4012279.00**	26.63***	24.03***	539.80***	7.86
Environments	2.00	24862880.00***	222.91***	1245.20***	5119.45***	3.81
Var.* Env.	42.00	1505117.25	4.30**	9.08**	213.83	8.13
Pooled Error	126.00	342820.75	1.12	5.11	55.10	2.18

*Significant at P=0.05 **Significant at P=0.01.

divergence (evaluated based on the genetic distance between individuals) is a predictive feature that allows for the identification of crosses with a higher probability of success. Specifically, the evaluation of divergence can identify those crosses that will optimize heterosis while avoiding undesirable features. In the prediction of genetic divergence between genotypes, multivariate methods, such as principal component analysis and canonical variables are very useful tools.

The goal of this study was to evaluate the stability and genetic divergence among 20 experimental hybrids for the choice of suitable parents of new population. We also aimed to strengthen the further research strategies for predicting the high yielding stable and divers parents (single cross hybrids) for double cross hybrids through estimates of genetic divergence.

MATERIALS AND METHODS

The present experiment was carried out at the Maize Breeding Research Station (MBRS), Poonch (India) situated between 33°-25' to 34°-01' N latitude and 73° -58' to 74° -35' E longitude, at a height of 3300 MSL and bounded by Kashmir valley and line of control with Pakistan. The experimental materials consist of 22 experimental hybrids developed at MBRS, Poonch. The hybrids are moderately resistant to most of the prevailing diseases and insects. The experiment was conducted in randomized block design for three years, that is, 2009, 2010 and 2011 following the recommended agronomical practice for maize and physiological data, which was recorded from mean of five randomly selected plants from each entry. The stability among the hybrids was estimated following the method described by Eberhard and Russell, 1966. To study the genetic divergence among the hybrids used in present experiment and to know the fluctuation in clustering pattern, hybrids were calculated by canonical (Vector) and non-hierarchical Euclidean methods of divergence estimation. The D2 values were calculated by using the method described by Mahalanobis (1936). Genetic divergence analysis using canonical (vector) method is a sort of multivariate analysis where canonical vector and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively, were derived (Rao,1952). Non- hierarchical Euclidean cluster analysis (Beale, 1969; Katyal et al., 1985) was conducted using a computer software package (Windostat version 8.5).

RESULTS AND DISCUSSION

The combined analysis of variance across locations and years showed highly significant differences among

locationns (L) and genotypes (G) and their interaction (G x L). The maximum grain yield variation was observed due to genotypes whereas the environments play highest significant role in variation due to days of tasseling and maturity (Table 1). Genotype environment interaction was highly pronounced in variation for shelling percentage among the genotypes (Dabholkar, 1999). The knowledge about the extent of fluctuations of yield and yield attributes over environments is very important in identifying genotypes, which are widely adapted.

Grain yield is a quantitatively inherited character and there is considerable interaction between genotypes and environments. Some of the crop varieties are widely adapted, whereas others are not. Because of the interactions between genotypes and environments, yield of genotypes tested across years vary and it is a problem for breeders to identify varieties that consistently give high yields in locations with diverse environmental conditions. Kang and Gorman (1989) reported that G x E interactions significantly reduced correlations between phenotypic and genotypic values. The results showed the hybrid PHM 12 was the highest among the others for grain yield, followed by PHM 34, PHM 14, PHM 10 and PHM 31 (Table 2). According to the joint regression model, the most stable hybrid with high mean yield and lowest S2 di value (0.999) was PHM 10 and its regression coefficient (bi) is 1.50, which can be an indication of its responsiveness to favourable environments (Table 2). Whereas the second most stable hybrid is PHM 31 with bi value 1.11, s2di 0.994 and high mean grain yield.

According to the model, the third most stable hybrid was PHM 34 with bi = 0.722, S2 di = -0.993 and mean yield 6.4 t ha-1 followed by PHM 23 and PHM 35 with bi value 1.73 and 0.79 respectively, both the hybrids exhibited high grain yield. Although the mean yield value of PHM 34 is less than PHM 12, the performance of the hybrid under unfavourable environments can be predicted. The evaluation showed that these single cross hybrids have high yield potential and are well suited in the prevailing agro-climatic condition. Further, the hybrids may be used as source material for double cross hybrids as they showed stable yield and desirable agronomic characteristics, with large proportions of favourable loci previously selected. From the point of selecting the hybrids (parents) for hybridization, which are divergent

Table 2. Estimation of stability parameters for grain yield in maize hybrids.

S/N	Variety	Mean (μ)	Regression (b_i)	Deviation ($S^2 d_i$)
1	PHM 10	5440.600	1.508	1.000
2	PHM 11	5146.600	-0.057	0.633
3	PHM 12	6586.500	1.114	0.317
4	PHM 13	4363.700	1.433	0.979
5	PHM 14	5506.700	0.965	0.534
6	PHM 15	5196.300	2.034	0.997
7	PHM 16	3929.000	0.910	0.945
8	PHM 17	4650.200	1.968	0.972
9	PHM 18	3373.900	0.855	0.997
10	PHM 20	2266.400	0.177	0.422
11	PHM 22	5192.400	0.745	0.930
12	PHM 23	4414.300	1.732	0.992
13	PHM 24	4929.200	1.984	0.995
14	PHM 25	2123.200	1.511	0.991
15	PHM 27	3273.500	0.383	0.018
16	PHM 30	3758.300	1.208	0.989
17	PHM 31	5271.500	1.120	0.994
18	PHM 34	6445.000	0.722	0.994
19	PHM 35	5100.900	-0.791	0.960
20	PHM 39	3546.200	-1.105	1.000
21	Check (Kh. 612)	4204.700	1.785	0.894
22	Check (Kh. 517)	4966.000	1.800	0.967

enough for the character of interest, estimation of the genetic distance is most important. The present estimation of genetic divergence gave clear idea about the diverse nature of the population. Wide range of variation was observed in cluster mean performance for most of the characters studied. All the genotypes were grouped into five clusters, indicating the presence of diversity for different traits.

On the basis of results obtained from the present study, high degree of genetic divergence was observed among the hybrids under study (Liu et al., 2006). It is evident as more number of cluster (five) were formed by 22 hybrids (Figure 1) and high range of inter and intra cluster distance. Singh et al. (2005) also found wide range of variability in maize genotypes in his experiment. The more the diversity of parents, the greater the chances of obtaining high heterosis. (Williams and Hallarver, 2000). More et al. (2006) also reported more number of clusters and genotypes within the clusters in his experiments. Cluster 3 had maximum numbers of hybrids and cluster 2 and 5 had minimum number of hybrids (Figure 1). The maximum inter cluster distance was observed between cluster 1 and 5, and minimum was between cluster 3 and 5 (Table 3). The maximum intra cluster distance was noticed between hybrids of cluster 5 whereas it was minimum in cluster 2 (Table 2). The result indicated that the hybrids in different clusters differ largely and marginally in their genetic architecture as per their maximum and minimum intra cluster distance, respectively.

Similar experiment was also carried out by Castanon et al. (1999) and Chen et al. (2007) who were of the opinion that geographical and genetic diversity exhibited no correspondence between them as hybrids from one and different origins were grouped together. This might be due to free exchange of genetic material from different regions. These findings were similar to the findings of Yin et al. (2004).

Principal factors were carried out using the principal component (PC) method for factor extraction. Differentiation among populations occurs in stages, or in other words in different axes of differentiation which accounts for total divergence (Crossa, 1990). Theoretically, many axes of differentiation can be envisaged as there are characters contributing to total variation, but it is not absolute. It is possible that most of the variation is accounted for by the first two or more axes of differentiation (Liu et al., 2006).

In the present investigating only the first three principal components showed eigen values more than one and cumulatively they explained 81.28% variability (Table 4). The first principal component explained 39.49% of the total variation and the second and third principal components explained 25.66% and 16.13% variation, respectively. The first principal component ($\lambda 1$) absorbed and accounted for maximum (39.49%) proportion of
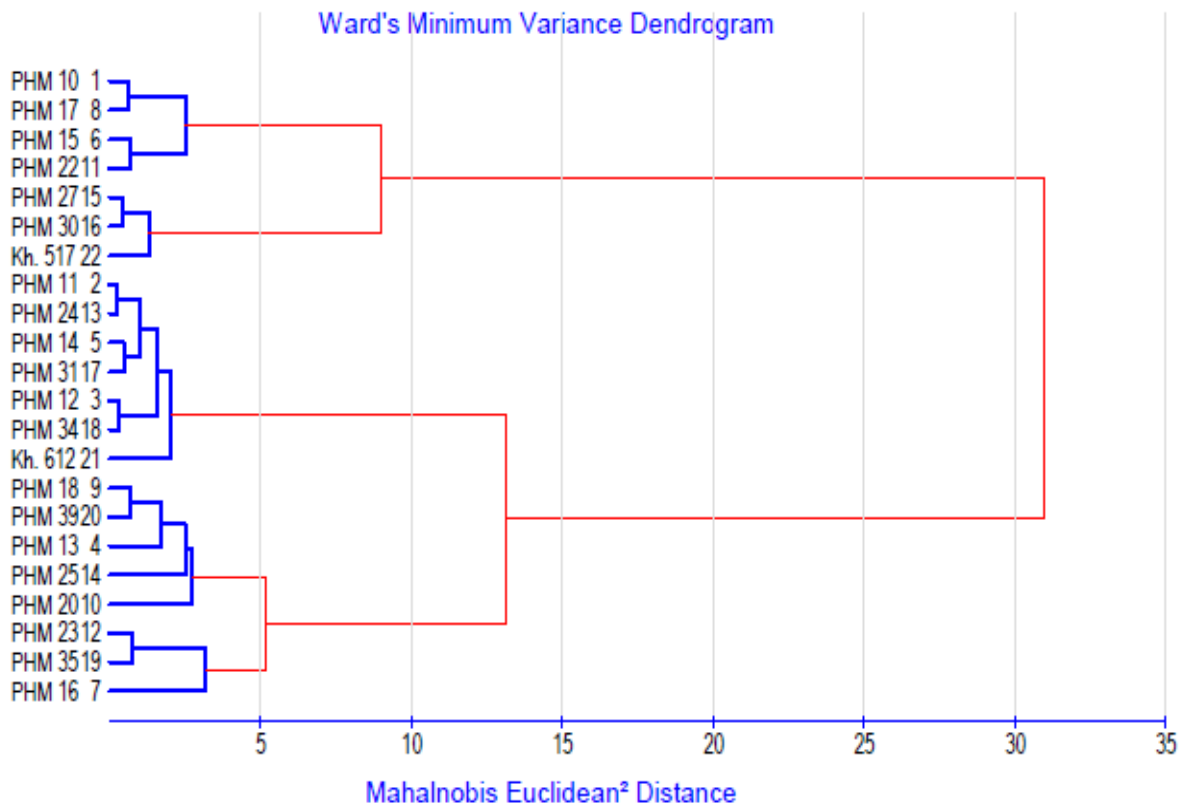
Figure 1. Clustering pattern of maize hybrids by Euclidean method.

Table 3. Mean inter and intra cluster distance among five clusters in hybrid maize by Euclidean method.

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster
1 Cluster	2.497	6.783	7.61	15.616	18.370
2 Cluster		1.752	8.062	7.413	13.540
3 Cluster			1.907	7.066	5.373
4 Cluster				3.817	5.590
5 Cluster					3.911

variability and the remainder accounted for progressively lesser amount of variation (25.66 and 16.13) for $\lambda 2$, $\lambda 3$ and $\lambda 4$, respectively. The study through canonical analysis revealed that on the basis of two axes, the cumulative percentage of variation absorbed by $\lambda 1 + \lambda 2$ was 65.15% whereas there are three effective axes Z1, Z2 and Z3 where $\lambda 1 + \lambda 2 + \lambda 3 = 81.28\%$.

In Vector 1 shelling percent with element value 0.523; in Vector 2 grain yield with element value 0.780 and in Vector 3 days to maturity with element value -0.780, contributed maximum to the total divergence at primary, secondary and tertiary axes of differentiation based on canonical vectors Z1, Z2 and Z3, respectively. Liu et al. (2006) also reported the similar types of findings in his

experiment. The character contributing maximum to the divergence should be given greater emphasis for deciding the type of cluster for purpose of further selection and the choice of parents for hybridization. Genetic divergence between genotypes was measured in terms of spatial distance and resulted in formation of three dimensions (3D) based on three PCA scores ($\lambda 1$, $\lambda 2$ and $\lambda 3$ graphs) as depicted in Figure 2. Three principal factor scores were used to plot all the 22 hybrids using PCA1, PCA2 and PCA3, that is, 3D plot which accounted for most important component traits namely: shelling percent, grain yield and days to maturity. A similar type of study was also carried out by More et al. (2006), who reported that leaf area per plant, plant height

Table 4. Canonical vectors which supply best linear function of variates, value of canonical roots and percentage of variation absorbed by respective roots.

Parameter	1 Vector	2 Vector	3 Vector
Eigene value (Root)	1.974	1.283	0.807
% Var. Exp.	39.487	25.663	16.135
Cum. Var. Exp.	39.487	65.149	81.284
Grain yield (kg/ha).	0.151	0.780	0.323
Days to tasselling	0.514	0.453	-0.263
Days to maturity	-0.417	0.264	-0.780
Plant height (cm)	0.515	-0.300	0.011
Shelling %	0.523	-0.164	-0.468
Gennotype	Vector 1	Vector 2	Vector 3
1PHM 10	10.620	3.399	-12.184
2PHM 11	9.927	3.049	-11.905
3PHM 12	9.925	3.386	-11.703
4PHM 13	9.931	2.504	-11.544
5PHM 14	9.813	3.079	-11.710
6PHM 15	10.435	3.142	-11.808
7PHM 16	9.440	2.866	-11.776
8PHM 17	10.535	3.187	-12.285
9PHM 18	9.808	2.443	-11.930
10PHM 20	9.487	2.245	-12.064
11PHM 22	10.440	3.018	-11.995
12PHM 23	9.363	2.727	-11.730
13PHM 24	9.757	2.985	-12.018
14PHM 25	9.435	2.208	-11.849
15PHM 27	10.314	2.437	-12.086
16PHM 30	10.477	2.549	-12.055
17PHM 31	9.514	3.041	-11.769
18PHM 34	9.912	3.307	-11.889
19PHM 35	9.453	2.769	-11.707
20PHM 39	9.721	2.693	-12.098
21Kh. 612	9.848	3.031	-11.937
22Kh. 517	10.422	2.670	-11.715

and days to 50 percent flowering were the major contributor towards divergence, while studying forage maize. Amongst 22 maize hybrids, studied in the present study PHM 10 exhibited great extent of genetic diversity on the basis of 3D diagram based on PCA scores and Euclidian distance matrix, which reflected it as most diverse genotype for PHM 20 followed by PHM 25, PHM 23, PHM 35, PHM 31 etc. Whereas, the minimum genetic was observed between PHM 14 and K 612 (Figure 2). The hybrids, PHM 10, PHM 23, PHM 35 and PHM 31 showed significantly high stability and divergence for grain yield. Expectations for different combinations of quality characters along with yield and yield attributes should be considered as selection criteria for desired improvement.The similar type of work have also been done by Mishra and Dash (1997) who had found similar response of breeding material towards stability and divergence.

Conclusion

Estimation of combining ability of parents is not always sufficient to reveal which combination should be used in breeding crosses. Instead analysis related to stability and genetic divergence of hybrids should be assessed together in evaluating potential crosses particularly for double cross hybrid. Considering the estimation of stability and genetic diversity together in the present investigation, both directional crosses namely PHM 10 x PHM 23, PHM 10 x 35 and PHM 10 x PHM 31 may be recommended which can throw out superior segregants in hybridization.
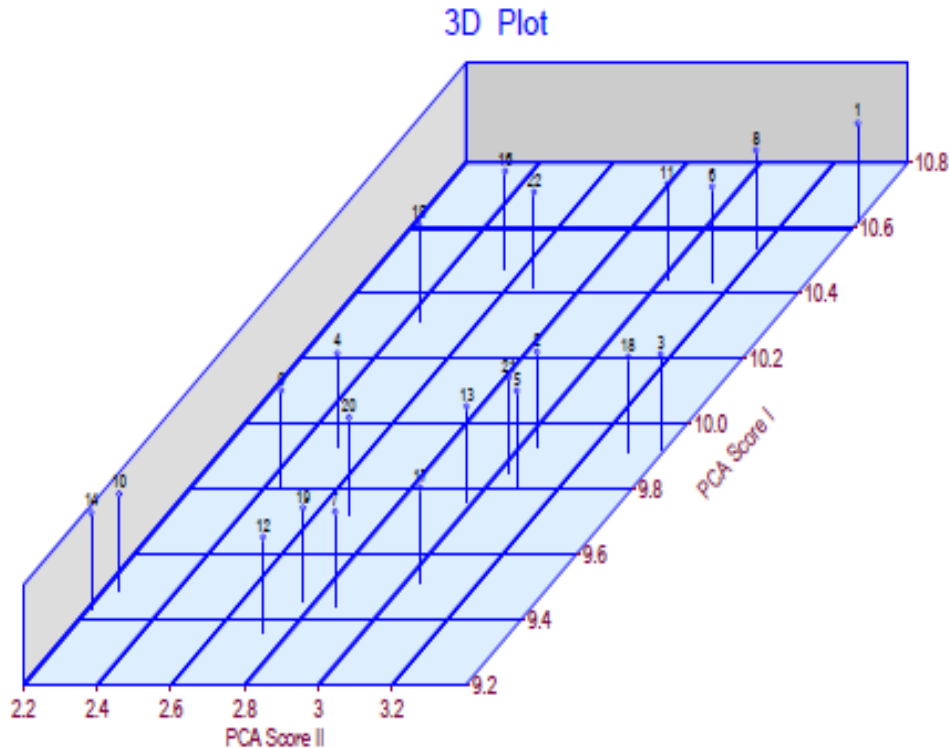
Figure 2. Three- dimensional representation of hybrids using 3 principal components based on canonical variates

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Estimation of orange yield in citrus orchard based on digital photography

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The citriculture is an activity that has relevance in the Brazilian agribusiness. Many researchers have sought to develop methods for oranges yield estimation, however few are simple and accurate. Thus, the aim of this work is to develop a practical method to estimate number of fruits and fruit´s mass per plant of sweet orange cv. "Valência" [*Citrus sinensis* (L.) Osbeck] orange grafted on 'Rangpur' lime (*Citrus limonia* Osbeck) rootstocks with North-South orientation using digital camera. Were taken 2 photos per plant in orange orchard, one for each face (southeast and northeast). The photos were visually analyzed by 3 assessors. In the harvest we quantify the number of fruits (NFR), medium fruits mass (MMF, g) and fruit´s mass per plant (KFP, kg plant^{-1}). Were tested the number of photos needed to the estimations and what face could show greater accuracy. The number of fruits per plant can be estimated with high precision using 10 photos taken of the Northeast face of the trees. The estimation of the fruit´s mass per plant it is also possible using 11 photos taken of the Northeast face of the trees.

Key words: Modeling, digital camera, Citrus, linear regression.

INTRODUCTION

The citriculture is an activity that has relevance in the Brazilian agribusiness (Brito et al., 2012). The citrus is considered one of the globalized segments of agribusiness, placing Brazil as the greater citrus producer, world market leader (Maria et al., 2013; Struiving et al., 2013).

The "Valência" orange shows great prominence in the Brazilian citrus industry (Pereira et al., 2014), because shows quality and fruit juiciness (Auler et al., 2009), high levels of organic acids, soluble solids and of yield (Pereira et al., 2014). The prior knowledge of yield level, before harvest, is essential to support farmers in decision

making (Triboni and Barbosa, 2004). The estimated yield provides valuable information in planning for agribusiness and is an important tool in farm sustentability (Zhang and He, 2013; Oliveira et al., 2013).

The estimated yield in orange is performed by direct and indirect methods (Triboni and Barbosa, 2004). In the direct method does field sampling to quantify the components of yield (Pino and Amaro, 1986; Ribeiro et al., 2008). However, in the indirect method we seek quantitative relationships between climatic (Aparecido et al., 2014), physiological and yield variables of orange, establishing simulation models to predict the growth,

Figure 1. Photography of the face southwest in the orchard of orange "Valência".

development and yield (Mantovani et al., 2010; Oliveira et al., 2013). Paulino and Volpe (2001) using linear regression statistical methods related the yield orange "Pera" with some meteorological variables. Martins and Ortolani (2006) developed agrometeorological models to estimate the yield of "Valência" orange in location Matão, Sao Paulo and observed good performance of the model ($R^2 = 0.81$) when used phenological periods of pre-flowering, flowering and fruiting. The prediction of number of citrus fruits can be made from the number of reproductive structures in orange, Rolim et al. (2008) in a work conducted in Cordeiropolis, Sao Paulo, with an orange "Valência" showed a high precision values ($R^2 = 85$) and low error (MAPE = 15.2%).

Precision agriculture has been an important and efficient tool to estimate yield in Citrus, using techniques as: aerial photography (Whitney et al., 1999), multi-spectral image (Annamalai and Lee, 2003) and ultrasonic sensors (Zaman et al., 2006). However, obtaining the information by these methods is considered expensive and with quality variable mainly because the sensor sensitivity and the difficulty of processing data (Zaman et al., 2008). Thus, the use of other methods to estimate the yield of citrus, as the use of photography from digital cameras is becoming interesting.

It is a method used in some crops, as Wheat (Pan et al., 2007), rice (Swain et al., 2008), blueberry (Zaman et al., 2008) and apple (Aggelopoulou et al., 2011). The main advantages are the low cost and small time of operation (Swain et al., 2007). Many researchers have been developing methods for estimation of yield orange; however few are simple and accurate. Thus, the aim of

this work is to develop practical method to estimate number of fruits and fruit´s mass per plant of the "Valência" orange grafted in lime "Cravo", using digital camera.

MATERIALS AND METHODS

The experiment was conducted in a 16 years old orchard of orange "Valência" [*Citrus sinensis* (L.) Osb.] grafted in lime "Cravo" (*Citrus limonia* Osb.) spaced 8 × 5 m, with North-South row orientation, located in Cordeiropolis, State of Sao Paulo, Brazil (22° 32'S , 47° 27'W longitude and altitude 639 m). Plants were grown under natural environmental conditions, that is, non-irrigated and exposed to natural variations of solar radiation, air temperature and humidity. The soil is classified as rhodic haplustox (Ramos et al., 2010) and the predominant climatic classification following Thornthwaite (1948) is B'₁rB'₃a, humid mesothermic, without or with little water deficit and accumulated potential evapotranspiration in summer lower than 48% of the total annual.

The average dimensions of the trees were 3.7 m of height, 4.7 m diameter and 2.3 leaf area index producing canopy of about 300 m^3. In March of the year 2012, we photographed 56 photos of 28 orange in the orchard (Figure 1), 2 photos per plant one for each face (Southeast and Northeast), using a digital camera with 8 megapixels. The number of fruits per plant (NFR) was visually evaluated by photographs that were quantified by 3 assessors (repetitions). After, the fruits were harvested to obtain the real values. The harvest was in December 12, 2012, quantified number of fruits per plant (NFR), medium fruits mass (MMF, g) and Fruit´s mass per plant (KFP, kg plant^{-1}) by weighing process.

The minimum number of plants necessary for accurate estimation of NFR in the orchard was determined. We tested NFR and KFP with the re-sampling data increasing the number of plants (photos) up to 28. For each sample we made a linear regression analysis calculating the accuracy by the mean absolute percentage error (MAPE) and the precision by the adjusted coefficient of
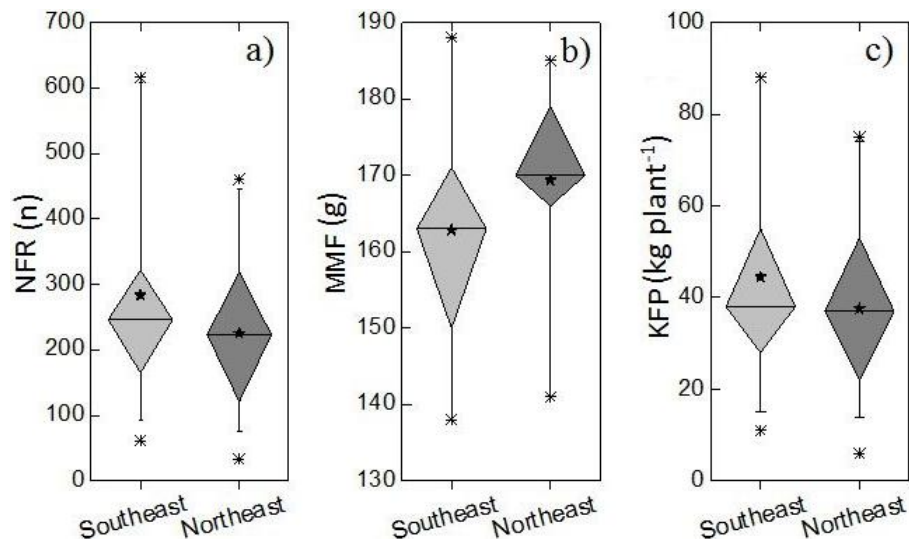
Figure 2. Observed number of fruits (NFR) per plant (a), medium fruits mass (MMF, g) (b) and fruit´s mass per plant (KFP, kg plant^{-1}) of orange "Valencia" in Cordeiropolis, SP. The horizontal bar mean the median; the lozenge means 95% of the data, the upper and botton star means the maximum and minimum absolute values and the vertical bars 99% of the data, the stars and asterisks means the average and absolute values, respectively.

determination (R^2 adjusted, Cornell and Berger, 1987) for Northeast and Southeast face and sum of both among observed and estimated values (Equations 1 and 2, respectively). We used the standard deviation values to analyze the stabilization of the estimations. The face of the plant canopy that provided the best stabilization of the precision and accuracy with minimum number of photos (NPH) was the selected to develop the estimation method:

$$MAPE = \frac{\sum_{i=1}^{NPH}\left(\left|\frac{Yest_i - Yobs_i}{Yobs_i}\right| \times 100\right)}{NPH}$$

$$R^2\ adjusted = \left[1 - \frac{(1-R^2)\times(NPH-1)}{NPH-k-1}\right]$$

Where, Yest$_i$: estimated value; Yobs$_i$: observed valuee; Y$_{est-C}$: variable estimated by linear regression between the observed value (Yobs$_i$) and estimated (Yest$_i$); k: number of independent variables in the regression.

RESULTS AND DISCUSSION

The Southeast face in the "Valência" orange showed 21% more number of fruits (NFT) than Northeast face (Figure 2a), providing greater fruit´s mass per plant (KFP) (Figure 2c) and lower MMF (Figure 2b). As Hafle et al. (2009) stated this inverse relationship between NFT, KFP and MMF occurs due the font-drain relationship in the plant. Fewer fruits reduce competition between them promoting an increasing of MMF.

The face "Valência" orange selected to estimate the NFR was the northeast, because the estimated values (photo) showed higher precision and accuracy and

stabilization occurred with lower number of photos (NPH) in comparison to the other faces facilitating the applicability of the method. The stabilization in the northeast face occurred from 7 NPH showing an average precision of 0.6 (Figure 3a). The southeast face and sum of the faces (NE+SE) the stabilization occurs only after 20 and 17 NPH, respectively (Figure 3b and c).

Evaluating the accuracy (MAPE) of estimated values in relation to those observed, the northeast face showed higher stabilization as well. This occurred from 7 NPH, showing an average accuracy of 35% (Figure 3d). The estimated values of southeast face and sum of the faces, do not demonstrated stability until the maximum NPH used in resampling (28 photos) (Figure 3e and f).

The validation analysis for NFR models using northeast faces showed distinct values of accuracy (MAPE). For example the model with 7 NPH could estimate NFR with an error of 8.1%, and the model with 10 NPH had 3.7% of error (Figure 4). Then we understand that a minimum of 10 photos of the Northwest face should be taken for practical applications.

The model selected for estimating NFR was y = 4.189x - 437.8 calibrated with high precision with R^{2}adj = 0.96, showing a significant linear regression (p<0.01) (Figure 5a). The same model tested with independent data showed high accuracy with MAPE = 4.0%, showing that is possible the estimation of the NFR "Valência" orange in function of the 10 NFR by northeast face photos. For example, in the validation of this linear model an average of 330 fruits per plant (final value) there is an error of ± 12 fruits, a low value of error in field conditions.
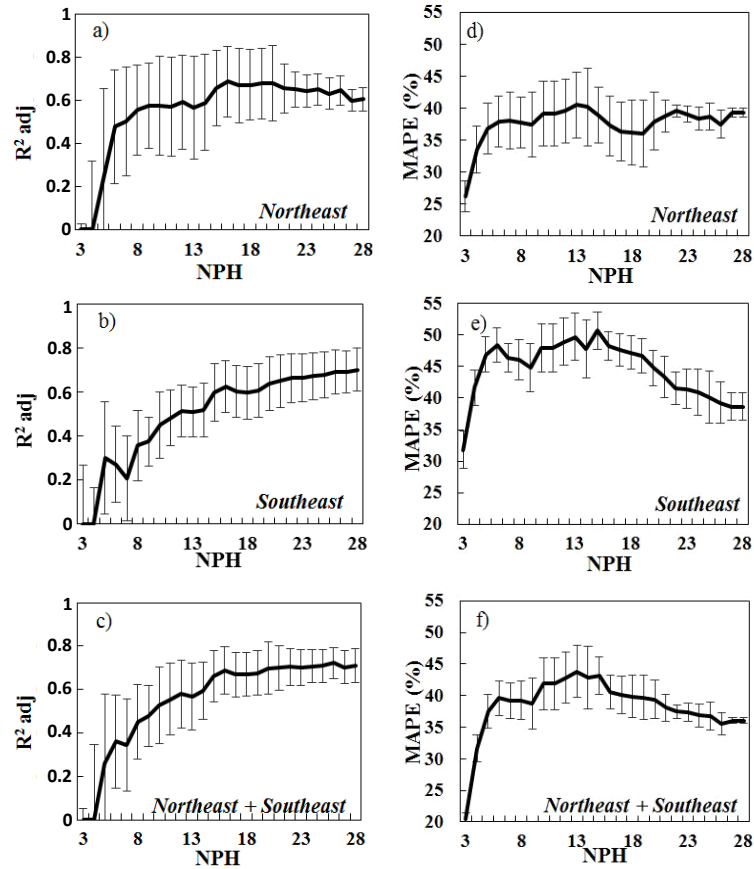
Figure 3. Evaluation of the precision (R^2 adjusted) and accuracy (MAPE) by resampling in models of number of fruits (NFR) estimation in function photos number (NPH) taken in the northeast (a), southeast (b), and the sum of the northeast and southeast faces (c). The vertical bars mean the standard deviations of the R^2adj of different samples.
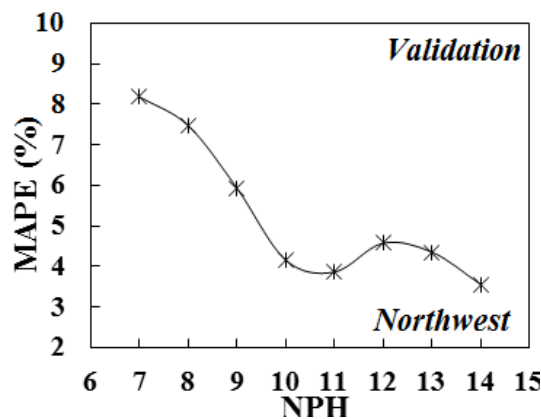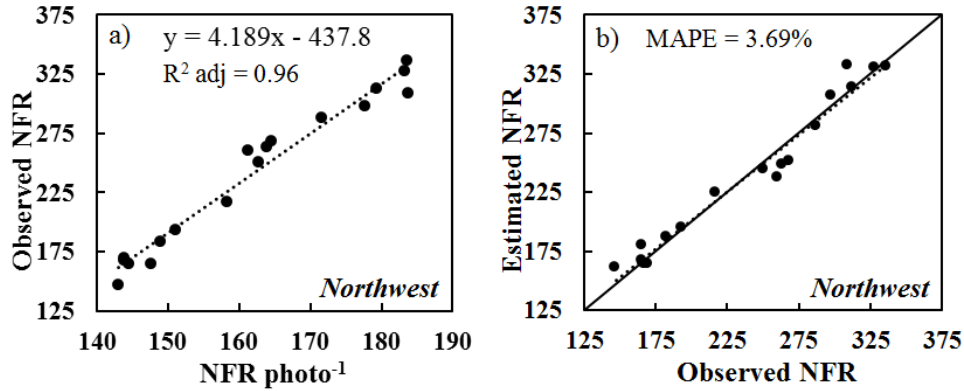

**Figure 4.** Validation of the accuracy of models developed to estimate number of fruits (NFR) with different number of photos (NPH).

The Northeast face was used as well for medium fruit mass per plant (KFP) estimation, because estimated values showed a stabilization of R^2adj with a minimum NPH (Figure 5). The stabilization occurred with 8 photos of the northeast face with average values of R^2adj = 0.65 (Figure 6a). In the other faces, the stabilization occurred
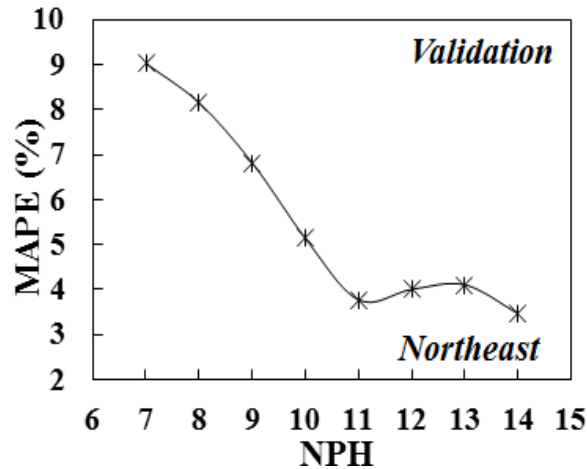
Figure 5. Calibration of the model to estimate the number of fruits per plant (NFR) in relation to NFR per photos (a) and validation of the model performance (b).
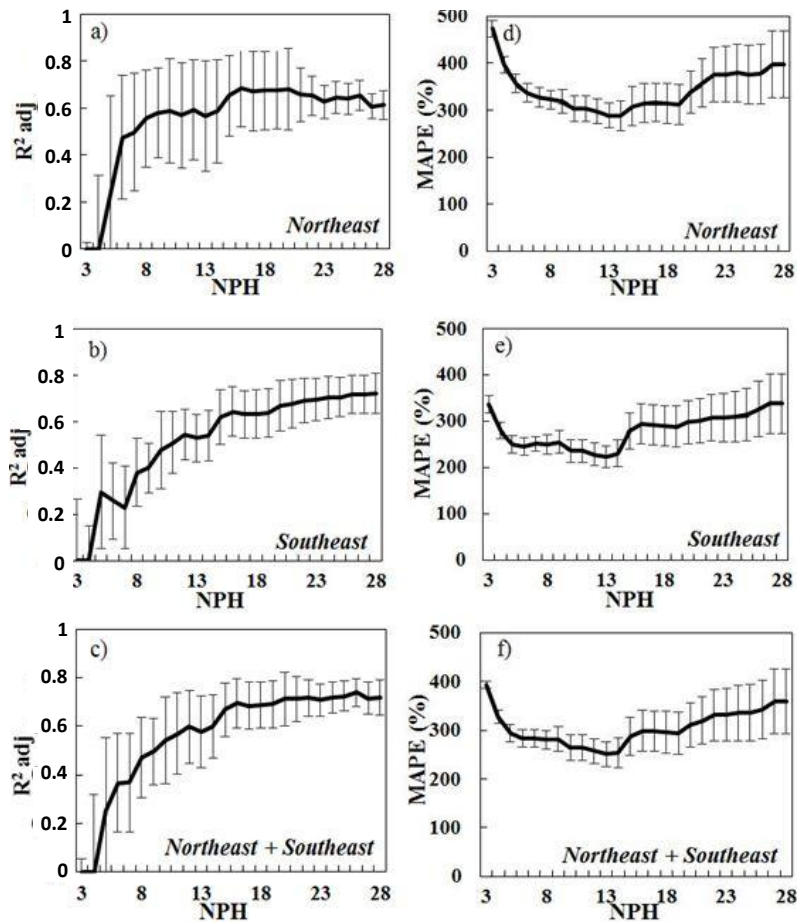

Figure 6. Evaluation of the precision (R^2 adjusted) the accuracy (MAPE) by resampling in models of average fruit´s mass per plant (KFP) estimation in function photos number (NPH) taken in the northeast (a), southeast (b), and sum of the northeast and southeast faces (c). The vertical bars mean the standard deviation of different samples.

with 21 and 17 NPH for southeast and sum of the faces, respectively (Figure 6b and c). The MAPE values in this

analysis had a different pattern in comparison to NFR and the values where higher up to 400% (Figure 6d, e

Figure 7. Evaluation of the accuracy of models developed to estimate fruit´s mass per plant (KFP); NPH = number of photos.
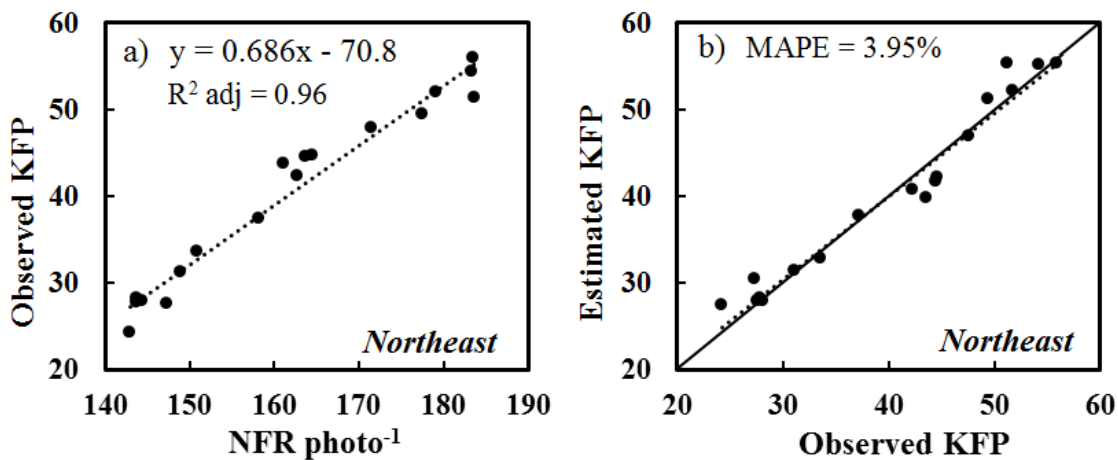
Figure 8. Model to estimate the number of fruits per plant (KFP) in relation NFR per photos (a) and evaluation of model performance (b).

and f). The minimization of MAPE was between 8 and 13 NPH. The higher values were not a problem because this analysis was used solely to find the better NPH for a following regression adjustment.

The validation analysis for KFP models using northeast faces showed high sensibility to the NPH (Figure 7). For example the model with 7 NPH could estimate KFP with an error of 9.0%, and the model with 11 NPH had 3.9% of error (Figure 7). Then we understand that a minimum of 11 photos of the Northwest face should be taken for practical NPH estimations.

The model selected for estimating KFP was y = 0.686x - 70.8 calibrated with high precision with R^2adj = 0.96, showing a significant linear regression (p<0.01) (Figure 8a). The same model tested with independent data showed high accuracy with MAPE = 3.95%, showing that

is possible the estimation of the KFP "Valência" orange in function of the 11 NFR by northeast face photos (Figure 8b). An error of 3.95% for KFP indicates in a plant with 55 KFP there is a variation of ±2.1 kg, a low error value for an adult plant.

Conclusion

This method is a valuable tool to estimation of the number of fruits per plant fruit´s mass per plant. The number of fruits per plant can be estimated with high precision using 10 photos taken of the northeast face of "Valência" orange with a maximum error of 3.7%. The estimation of the fruit´s mass per plant it is also possible with a maximum error of 3.9%, using 11 photos taken of

the northeast face of "Valência" orange.

Conflict of Interest

The authors have not declared any conflict of interest.

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