

Original Research Article

Effects of nitrogen levels and Arbuscular mycorrhizal fungi on biomass production, mineral nutrition, sugar and total phenolic content of two *zea mays* cultivars.

ABSTRACT

The effects of two nitrogen levels and AM Fungi inoculation on nutrition assimilation and some biochemical compounds of two *Zea mays* varieties was study. Plants varieties (CMS8704 and POP66RS) were grown in pots with two nitrogen levels and AM fungi inoculation for 82 days. Plants growth under Low N condition showed significant reduction in biomass production, N and P uptake. POP66RS cultivar proved to be more efficient than CMS8704 under nitrogen deficiency environment, which was reflected by increased chlorophyll rate and carbohydrates content. However, under AM fungi CMS8704 variety show better performance than POP66RS variety. Significant increase in plant dry weights, P and N uptakewere recorded following AM inoculation. AM fungi positively influence phenolic compounds while it decrease the soluble carbohydrates and total chlorophyll under low and high N, for the two cultivars. Evaluating such parameters in plant during it growth could be further exploited for maize selection.

Keywords: Nitrogen, Maize varieties, mycorrhizal fungi, Carbohydrates and Phenol compounds.

1. INTRODUCTION

Maize (*Zea mays* L.) is the most important crops in the world after wheat and rice [1]. It is a major item in the diet of many tropical people. In Cameroon, this plant can growth in all the five local ecological zones and is now a cash crop for many farmers [2]. Local demand of maize continuously increases within decays for reasons like high demand particularly from a large

16 scale local buyers including. They include: feed industry, breweries, a larger section of growing
17 urban population [1].

18 The production of this cereal is dominated by small holder farmers, who always used traditional
19 manual methods generally are fraught with drudgery and a lot of problems, with simple low input
20 technology, resulting in low land and labour productivity [3]. The absence of resistant or tolerant
21 local maize varieties to rust diseases is a limiting factor of maize production [1]. Pest and
22 diseases are important natural factors limiting the production of maize in several cases,
23 accounts for 100% losses [1]. Those constraints are highlighted in many local regions by low
24 nutrient capital in soil, erosion risks, low pH with salt toxicity, high phosphorus fixation, N
25 depletion policy and loss of soil biodiversity [4,5]. Such conditions generally lead to bad feeding
26 of plant and low yield. Nitrogen is one of the few nutrients in terrestrial ecosystems that may
27 limit growth of plants [6]. It is necessary for many functions in plant such as chlorophyll, proteins
28 and amino acids synthesis [7]. Nitrogen (N) plays a vital role in the development of crop yield
29 and quality. It is a mobile element in the soil and hence under humid conditions is subjected to
30 leaching indicating the importance of appropriate N fertilization under such conditions [8]. N
31 deficiency results in accumulation of carbohydrates (sugars and starch) in the leaves, higher
32 levels of carbon allocated to the roots and an increase in root to shoot biomass ratio [9,10,11]. N
33 deficiency therefore affects, to various extents, primary photosynthesis, amino acid and sugar
34 metabolism, and/or carbohydrate partitioning between source and sink tissues [12,13,10].
35 Nitrogen excess does not increase yield or vegetative growth [14], but negatively affects
36 fruitquality or its derived products [15]. In current horticultural practice, N isoften applied in
37 higher amounts needed to ensure agood production [16]. N fertiliser recommendations are
38 traditionally based on soil N status and to a lesser extent on foliar analysis [17]. A rationalization
39 of fertilization is aprerequisite in the light of the factors up on which productivitydepends, namely
40 photosynthesis, translocation of assimilatesgrowth and production.

41 It is increasing recognize that ecosystem functioning could be regulated by groups of soil
42 microbes with special functional importances including mycorrhizal fungi [18]. Arbuscular
43 mycorrhizal (AM) fungi are soil fungi, developing symbiotic association with most terrestrial
44 plants under low and high nitrogen conditions. The symbiosis is often mutualistic based largely
45 on exchange of C from the plant and P delivered by the fungi [19]. Despite their implication in
46 phosphorus fertilisation of plant, AM fungi can mediate more than 50% of plant nitrogen uptake
47 [20,21], modification of phenolic compounds metabolism [22,23,24], proteins metabolism [25]
48 and amino acids pathway [26]. We suggest that AM fungi hyphae may contribute substantially to
49 plant N nutrition.

50 Plant phenolics and carbohydrates have received considerable attention in relation to stress
51 resistance. In fact, the metabolic pathways of these substances are interconnected: phenolic
52 pathways use products of carbohydrate metabolism as their precursors [27]. Phenolic
53 compounds are increased in response to adverse environmental conditions, which play an
54 important role in regulation of biochemical, physiological and molecular responses in plants [28].
55 These include effects on nitrogen assimilation, ion uptake, enzyme regulation, membrane
56 organization, photosynthetic carbon dioxide assimilation and nutrient deficiency in plants

[29,30,31,32]. Levels of some compounds related to secondary metabolism show a sensitive response to nutrient deficiency in plants [33,34]. Provision of nitrogen (N), either from organic or mineral fertilizers, has far-reaching consequences for the performance of plants at the biochemical, ecophysiological and ecosystem levels [35]. Nitrogen strongly affects the use of environmental resources (water, light), in part because its metabolites (amino acids) and their derivatives (enzymes and co-factors) are of pivotal importance for plant growth and physiology. Environmental conditions, such as fertility of the habitat, can affect the concentration of secondary compounds [36]. The plant growing in nitrogen-poor condition is thought to contain more secondary metabolites compounds than plants growing in a nitrogen-rich environment. According to the carbon/nutrient balance hypothesis [37] when nitrogen availability in the soil is low, the low resource availability limits the growth of the plants more than the photosynthesis, and plant allocates the extra carbon that cannot be used for growth to the production of carbon based secondary metabolites (CBSM).

The objective of this study was to examine the effects of two nitrogen levels and AM fungi potential on chlorophyll content, total soluble carbohydrates, total phenolics, N, P, of two Zea mays varieties.

2. MATERIAL AND METHODS

2.1 Seed material, AM inoculums and N fertiliser.

Seeds of two maize genotypes (CMS8704 and POP66-SR) and a mixture of three AM fungi strains (*Acaulospora tuberculata*, *Gigaspora margarita*, *Glomus intraradices*) were obtained from the Maize Breeding Program at the Regional Biocontrol and Applied Microbiology Laboratory of the National Institute of Agricultural Research for Development (IARD), Cameroon. These varieties were selected according to their nitrogen tolerance in poor nitrogen substrate: POP66SR had the highest N assimilation rate while CMS8704 had the lowest. The AM mixture were authenticated on many local grown plants as maize, cowpea, beans and groundnut. Two N rates (20N and 100N) were prepared from urea source, corresponding to 20 kg N ha⁻¹ and 100 kg N ha⁻¹ respectively. This consisted of adding 0.049 g and 0.246 g per plastic bag for the 20 N and 100 N respectively.

2.2 Substrate preparation, plant culture and treatment.

The experimental substrate was neutral Sand (pH 6.8) collected from Wouri River in Cameroon. The so collected Sand was, sieved at 2 mm and abundantly washed with distilled water to removed residual organic matter. The substrate was then used to filled experimental pot of three liters.

Seeds were sterilized by immersion in 5 % sodium hypochlorite for 10 min, rinsed four times with distilled water and kept for germination on wet filter paper in Petri dishes at 25°C for 72h. After the germination process, the seed were transplanted into plastic pots. Prior to the transplantation, AM fungi pots were inoculated with 200g *Acaulospora tuberculata*, *Gigaspora*

94 *margarita* and *Glomus intraradices* (1-1-1) mixture containing approximately 100 spores per
95 types. Non AM pots received the same quantity of autoclaved inoculums. The inoculums were
96 placed adjacent to each seeding root.

97 **2.3 Experimental design**

98 The experiment took place at the National Institute of Agricultural Research for Development
99 (I.A.R.D.) under control conditions. The experimental design was a randomized complete block
100 with 3 factors 5 treatments and 5 replicates. The first factor was N application with two levels
101 equivalent to 20N= 20 kg N ha⁻¹ and 100N= 100 kg N ha⁻¹. The second factor was two maize
102 varieties POP66SR and CMS8704. The third factor was AM fungi application: AM fungi
103 inoculation (M) and none AM fungi inoculation (NM). Plants were daily watered with distilled. In
104 addition, each treatment received Rorison's solution without nitrogen to supply nutrient
105 deficiency. Rorison's solution is composed with 1.5 KH₂PO₄ 3H₂O; 1.2 MgSO₄ 7H₂O; 1.2 Fe-
106 EDTA; 0.11 Mn SO₄ 4H₂O; 0.019 CuSO₄ 5H₂O; 0.02 Zn SO₄ 7H₂O; 0.14 H₃BO₃; 0.1 MoNa₂O₄
107 and 0.375 CaSO₄ g.l⁻¹. Plants harvested 82 days after planting for sampling analyses.

108 **2.4 Plant analyses**

109 Seventy five days after planting, total chlorophyll (%) was evaluated *in situ* in the third leaf of
110 each plant, by using chlorophyll meter (SPAD-502). Shoots and roots of each plant were
111 weighed separately. One g of fine root was used for evaluation of AM fungi colonization [38].
112 Remaining roots, together with the shoots were dried at 60°C for 72 h. The whole plants were
113 crushed and sub-samples used for the determination of P and N concentration. Sub-samples of
114 plant powder were digested with concentrated sulphuric acid at 480°C and analyzed for N and P
115 content. Analysis of P-uptake was done using the ammonium-molybdate blue method describe
116 by [39] while N content was done using method describe by [40].

117 **2.4.1 Determination of carbohydrates.**

118 One gram of fresh materiel was crushed and extracted in 5 ml of 80% ethanol. [41] and titrated
119 using the anthrone method of [42]. Pure analytical grade glucose (1mg/ml) was used as
120 standard.

121 **2.4.2 Determination of total soluble phenol compounds**

122 Total phenols were extracted according to the describe method of [43]. Half g (0.5 g) of plants
123 powder was ground and mixed with 25 ml of 70% methanol at room temperature for 45 min. The
124 methanolic extract was filtered with Whatman n°1 paper and the methanol was evaporated at
125 40°C under vacuum using a rotary evaporator. The aqueous phase obtained was adjusted to 25
126 ml with sterile distilled water, and depigmented by adding 12.5 ml of 40% ammonium sulphate
127 [(NH₄)₂SO₄], 0.35 ml of 80% orthophosphoric acid and 25 ml of petroleum ether. The ether
128 phases were discarded and the aqueous phases were extracted four times with 25 ml ethyl

129 acetate. The aqueous phases were discarded and the organic phases of the same sample were
130 combined, dried by addition of five g of magnesium sulphate (MgSO_4) and filtered after 5 min
131 with Whatman n°1 paper. The salt residue was discarded and the clear organic phase was dried
132 at 40°C under vacuum using a rotary evaporator. Total phenol content was determined
133 according to protocols established by [44]. Total phenol content was expressed in mg of
134 chlorogenic acid per gram of plants.

135 2.5 Data analysis

136 The data were subjected to analysis of variance (ANOVA) using Statistical Analysis System
137 Software (SAS) version 9.1. The statistical significance of the results was determined by
138 performing Turkey's test at $P < .05$. Pearson's correlation was used to establish the relationship
139 between pairs of variables.

140 3. RESULTS AND DISCUSSION

141 3.1 Results

142 3.1.1 Root colonization and biomass production

143 Non inoculated maize plants did not show any structure characteristic of AM fungi in roots for
144 the two cultivars. Colonization rate decreased significantly by increasing N level application. The
145 colonization rate of POP66SR variety, at 20N and 100N nitrogen levels were 60% and 50%
146 respectively and those of CMS8704 variety were 66.7% and 56.7% at the two N levels
147 respectively (Table 1).

148 Increase N level did not affect significantly Shoot and root dry mater of the two maize varieties
149 (Table 1). However, AM fungi inoculation significantly improved shoot and root dry matter of the
150 two maize varieties at each N level. This effect of AM fungi on shoot and root dry matter was
151 better for the POP66SR variety than the CMS8704 variety at the two N levels (Table 1).

152 Table 1: Effect of N levels and AMF colonization on root colonization, shoot and root dry matter
153 (SDM, RDM) of two maize cultivars, 82 days after seedlings.

Parameters	Root colonization	SDM	RDM
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	20N		100N		20N		100N		20N		100N	
	NM	M	NM	M	NM	M	NM	M	NM	M	NM	M
POP66SR	0.0 ^c	60.0 ^a	0.0 ^c	50.0 ^b	2.04 ^d	3.36 ^c	2.92 ^{cd}	6.06 ^a	1.70 ^{cd}	2.70 ^c	2.03 ^{cd}	9.70 ^a
CMS8704	0.0 ^c	66.7 ^a	0.0 ^c	56.7 ^b	1.28 ^e	4.23 ^b	1.75 ^{de}	2.38 ^d	0.90 ^d	5.40 ^b	1.4 ^{cd}	4.1 ^b

154 Means within columns followed by same letters are not significantly different at $P < 0.05$ NM:

155 none A.M. fungi; M: inoculated with A.M. fungi; POP66SR and CMS8704 maize cultivars; 20N

156 and 100N: lowest and highest N levels.

157 3.1.2 Chlorophyll rate, Nitrogen and Phosphorus uptake

158 Increasing nitrogen level raising chlorophyll content of the CMS8704 variety but causes a
 159 reduction of this content for the variety POP66SR. AM fungi significantly reduced chlorophyll
 160 content for the two maize varieties excepted for the 100N level of the POP66SR variety (Table
 161 2).

162 Increase nitrogen level significantly raising P and N concentration of the two maize varieties. AM
 163 fungi inoculation did not influence P and N concentration for the CMS8704 variety however, a
 164 significant increase of P content was observed for the POP66SR variety at the low N level
 165 following AM fungi inoculation. The N content was significantly affected at the two N levels of
 166 the POP66SR variety (Table 2). The lowest P content was record for the CMS8704 variety at
 167 the low nitrogen level without AM fungi inoculation, and the highest at the high nitrogen level for
 168 the POP66SR with AM fungi inoculation. The lowest and highest N concentration was record for
 169 the CMS8704 variety at the low nitrogen level without AM fungi inoculation and the high
 170 nitrogen level with AM fungi inoculation respectively (Table 2).

Table 2: Effect of N levels and AMF inoculation on chlorophyll rate, nitrogen (N) and phosphorus (P) uptake of two maize cultivars, 82 days after seedlings.

Parameters	Chlorophyll (%)				N(mg/plant)				P(mg/plant)			
	20N		100N		20N		100N		20N		100N	
	NM	M	NM	M	NM	M	NM	M	NM	M	NM	M
POP66SR	28.10 ^a	9.50 ^e	25.80 ^{bc}	27.0 ^{bc}	1.50 ^d	2.25 ^{bc}	2.09 ^c	2.60 ^{ab}	3.0 ^d	8.38 ^c	9.09 ^b	11.86 ^{ab}
CMS8704	18.50 ^d	10.86 ^e	24.05 ^c	19.13 ^d	1.37 ^d	1.55 ^d	2.38 ^{ba}	2.75 ^a	0.80 ^e	1.74 ^{de}	1.46 ^{de}	2.21 ^{de}

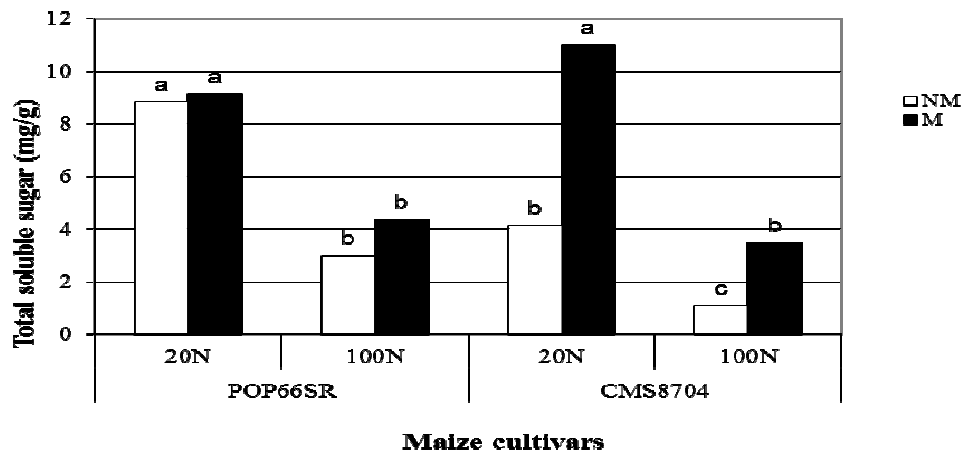
Means within columns followed by same letters are not significantly different at $P < 0.05$ N.M:

none A.M. fungi; M: inoculated with A.M. fungi; POP66SR and CMS8704 maize cultivars; 20N

and 100N: lowest and highest N levels.

3.1.3 Soluble carbohydrate, and total phenol contents

Total soluble carbohydrates of the two maize varieties leaves were significantly influenced by N levels application and AM fungi. There was neither varietal nor interaction effects observed in the study. The accumulation of soluble carbohydrates in our maize varieties was high under low nitrogen levels (20N) and low under high N level (100N). The same tendency was observed after AM fungi application (Fig. 1). The lowest sugar concentration was observed for CMS8704 maize variety at the high N level without AM fungi inoculation while the high soluble carbohydrate concentration was observed for POP66SR at the low N level and the CMS8704 at the same N level after AM fungi application. AM fungi application tends to increase soluble sugar concentration in all the varieties with significant effect in the CMS8704 variety (Fig. 1).



186

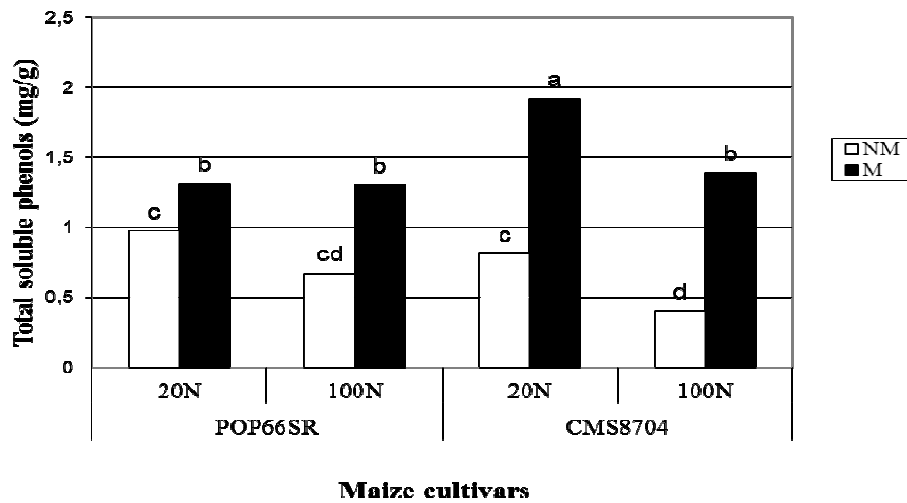
187 Figure 1: Effect of N levels and AMF colonization on total soluble sugar of two maize cultivars,

188 82 days after seedlings.

189 Barres with the same letters is not significantly different at P < 0.05. NM: none A.M. fungi; M:

190 inoculated with A.M. fungi; POP66SR and CMS8704 maize cultivars; 20N and 100N: lowest and

191 highest N levels.



192

193 Figure 2: Effect of N levels and AMF colonization on total soluble phenols of two maize cultivars,

194 82 days after seedlings.

195 Barres with the same letters is not significantly different at $P < 0.05$. NM: none A.M. fungi; M:
196 inoculated with A.M. fungi; POP66SR and CMS8704 maize cultivars; 20N and 100N: lowest and
197 highest N levels.

198 Soluble phenol concentration of the two maize varieties was significantly influenced by N levels.
199 When nitrogen level increase, the concentration of soluble phenolic decrease simultaneously.
200 AM fungi application increase significantly the total phenolic concentration of all the two maize
201 varieties at any nitrogen levels excepted for the 20N level of the POP66SR variety (Fig. 2). The
202 lowest phenolic concentration was observed for the CMS8704 variety at the high nitrogen level
203 (100N) without AM fungi inoculation and the highest at the low level (20N) of the same variety
204 after AM fungi application (Fig. 2).

205 **3.2 Discussion**

206 The colonization rate declined with increasing N level for the two maize varieties, indicating that
207 increase N level affect the growth of AM fungi (Table 1). It has been reported that addition of
208 various N application to soil inhibits hyphal growth with a subsequent decrease in the spread of
209 mycorrhizal colonization [45]. A number of other workers reported that root colonization
210 decreases in rich nitrogen soil [46, 47]. Our results further demonstrate that, under reduced N
211 fertilization (20N), inoculation with AM fungi significantly improved shoot and root dry weight of
212 the two maize varieties. The best response was observed with the CMS8704 variety (Table 1).
213 This observation is consistent with many others indicating the promotion of plant growth by AM
214 fungi under low nutrient availability [48, 49, 50]. We also noted that under high N level (100N),
215 maize SDM and RDW was significantly increased following AM fungi inoculation, with the best
216 performance observed for the POP66SR variety (Table 1). It seems possible that genetic factors
217 related to the maize varieties affect their aptitude to undergoing symbiosis with AM fungi and
218 subsequently their growth. This contrasted observation go in line with [51] and [45] who

219 hypothesized that, when plants are grown under optimal conditions growth promotion by AM
 220 fungi is unlikely, whereas under suboptimal conditions enhanced growth can be achieved.

221 Ours results showed that, nitrogen fertilization significantly influence chlorophyll content, N and
 222 P uptake of the two maize varieties. Increase N level significantly increase leaves chlorophyll
 223 rate of CMS8704 variety while it decrease in POP66SR variety (Table 2). Inoculation with AM
 224 fungi significantly influence leaves chlorophyll rates of the two maize varieties. Intrinsic genetic
 225 factors related to plant maize variety could justify this observation. It has been shown that the
 226 response of leaf photosynthesis is largely dependent on the leaf N content [52] and that N
 227 fertilization significantly affects Chlorophyll concentration of plants [53, 54, 55]. The N SDM
 228 content vary according to N application level for both maize varieties, with low amount obtain
 229 under low N level and the higher under high N level (Table 2). Shoot N increase is the result of
 230 the increase of N level application [52, 56]. A negative correlation ($r^2 = -0,89$; $p = .016$) was
 231 shown between AM fungi colonization and nitrogen content for POP66SR variety at 20N rate,
 232 however there were no correlation between the two parameter at 100N level. There was no
 233 correlation between the two parameters at the two nitrogen levels for the CMS8704 variety. This
 234 observation, can explain the implication of AM fungi and plant variety in the adaption of plant to
 235 low nutrient environment especially nitrogen. Decreased plant biomass production due to N
 236 shortage was associated with reductions in both nutrient assimilation [53] and leaf
 237 photosynthetic capacity [57]. There is also clear evidence that N deficiency induces decreased
 238 growth due to limitation of N and P nutrition within the whole plant [58 59, 60].

239 Different research workshave indicated that, AM fungi can substantially enhance the uptake of
 240 different nutrients, especially phosphorous (P) by the host plant through the extension of hyphal
 241 network [20, 61]. It has also been indicated that AM fungi can also influence the uptake of other
 242 nutrients necessary for plant growth and yield production including N [20, 61]. [21] indicated that
 243 more than 50% of plant N requirement was supplied by mycorrhizal association.

Our investigations also reveal that, N fertilization level has significant effects on carbohydrate and phenol leaves content. The leaves soluble carbohydrate and phenolic content was higher in limited N fertilization (20N) than in high N treatment (100N) for the two maize cultivars (Fig. 1). Many study showed that under inappropriate growth conditions such as drought, high salinities, low temperatures [62], nitrogen deficiency [25, 52, 63], there is a large accumulation of soluble sugars generally interpreted as an adaptive response of plant to such conditions [62]. Other clearly shows the increase of phenylalanine ammonia lyase (PAL) activity in plant leaf following N depletion resulting to the synthesis and accumulation of phenolic compound [64]. Probably those two compounds are used by plant to develop adaptation in environmental stress conditions, as they are higher in CMS8704 variety than POP66SR, suggesting the best adaptation capacity of POP66SR under low N condition. Inoculation with AM fungi stimulates the synthesis and accumulation of soluble carbohydrate under the limited N fertilization with remarkable effect on the CMS8704 variety (Fig.1). Positive significant correlation was observed between AM fungi colonization and phenolic compounds of the CMS8704 variety the two nitrogen levels ($r^2=0,96$; $P= .002$ and $r^2=0,98$; $P= .00$). This observation goes in line with other study showing the contribution of AM fungi to plant adaptation [65]. AM fungi also increase significantly phenol content at the two N levels for the CMS8704 variety at the two N levels while it affect less the content of this compound for the other variety. This is an additional argument supporting the high dependency of CMS8704 variety under low N fertilization condition.

4. CONCLUSION

N levels caused modification in leaf N and P content; Chlorophyll rate, soluble sugar and phenolic compound leaves content and root colonization by AM fungi, resulting in a lower of shoot and root dry matter accumulation of the two maize varieties. Those modifications are related to both plant genotypes and AM fungi inoculation. The parameters used in this experiment suggest that CMS8704 variety is more efficient than POP66SR variety under N

269 deficiency, which are highly AM fungi dependent. This approach could be further exploited for
270 cultivar selection.

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