Indian Journal of Ecology (2022) 49(2): 430-434
DOI: https://doi.org/10.55362/IJE/2022/3541

Manuscript Number: 3541
NAAS Rating: 5.79

Efficiency of Mycorrhiza Associated with *Piper mullesua* Plantlets under Acidic Soil Condition

Arundhati Bordoloi and A.K. Shukla[1]
Krishi Vigyan Kendra, Sivasagar-785 687, India, Assam Agricultural University
[1]Indira Gandhi National Tribal University, Amarkantak-484 887, India
E-mail: arundhatibordoloi@gmail.com

Abstract: The investigation was carried out to study the efficiency of arbuscular mycorrhizal fungi in up taking plant nutrients for the *Piper mullesua* plantlets grown in acidic soil condition. In the present study, *Piper mullesua* seedlings were infected with ten different strains of AM fungi and one without inoculation of AM fungi under controlled soil pH (4.5). Growth parameters and plant nutrients of AM infected plants were higher than those of non-mycorrhizal controls, which confirm the contribution of AM fungi. The two mycorrhizal fungal species *G. macrocarpum* and *G. hoi* were most efficient in pH 4.5 and encouraged the growth of the plantlets *P. mullesua*. The correlation coefficient between plant biomass and phosphatase activity was highly significant in case of *G. macrocarpum* associated with *P. mullesua* plantelts under acidic soil condition which confirms potentiality of the fungal inoculums in acidic soil.

Keywords: Mycorrhizal fungi, Acidic soil, pH, *Piper mullesua*, Phosphatase

Piper mullesua D. Don. (syn *P. brachystachyum* Wall ex Hook. fis indigenous to Arunachal Pradesh (India) and widely distributed in the Eastern Himalayan region at an altitude of about 600m to 1500m. It is an important medicinal plant belonging to the family *Piperaceae.* Roots and fruiting spikes are used in treating diarrhea, indigestion, jaundice, urticacia, abdominal disorder, horseness of voice, asthma, cough, piles, malaria fever, vomiting wheezing, chest conjestion, throat infection, worms and sinusitis. *Piper mullesua* is also considered as a rejuvenating plant. Myristicin, a 1,3-benzodioxole has been extracted from the hexane fraction of alcohol extract of fruit bearing inflorescence of *Piper mullesua* which has insecticidal properties (Srivastva et al 2001). Soil pH is known to have considerable effect on plant growth which influences the mobilization and availability of various essential and functional elements in soil (Ingrid et al 2002). Generally nitrogen and phosphorus are available to plants at a soil at pH range of 5.5 to 6.5 and on lowering the soil pH elements become unavailable to plants. Among the factors aluminum (Al) toxicity has been regarded as the main factor responsible for decreasing soil fertilities by decreasing availability of essential plant nutrients in acid soil. In neutral or alkaline soil solution, Al is present as harmless oxides and aluminosilicates (Martens 2001). However in soils below 5.5, the solubility of aluminum increases greatly and is released into the soil solution in the form of toxic ions to plants [$Al(OH)^{2+}$, Al^{3+} and $Al(H_2O)_6^{3+}$] (Kochian et al 2004, Roupheal et al 2015). To mitigate the negative effects of acid soils on plants, several management practices are typically implemented, such as lime application, P fertilization, and selection/use of Al-resistant plant genotypes. In addition, the inoculation/preservation of plants and soils with symbiotic arbuscular mycorrhizal (AM) fungi is another management alternative (Borie et al 2010).

Arbuscular mycorrhizal fungi are obligated symbionts of about 80 % terrestrial plants, some of them growing in soils with serious constraints (Smith and Read 2008). Arbuscular Mycorrhizal symbiosis allows a bidirectional interchange of nutrients and energy (Barea et al 2013, Smith and Read 2008). Essentially, host plants improve their water and nutrient absorption capacity, and fungi receive carbon compounds. The AM symbiosis is involved in plant adaptation to stressful soil conditions (Seguel et al 2013). Numerous studies have demonstrated that the Mycorrhizal fungi are widely distributed in acid soils and show relative tolerance to Al3+, Guo et al 2012, Fritz et al 2010). The formation of mycorrhizae could regulate the relationship between soil aluminum, phosphorus and plant, protecting roots from the Al toxicity (Vandamme et al 2013). However, mycorrhizal fungi differ in wide functional diversity among AM fungal genera and species in their capacity to alter the rhizosphere (Kelly et al 2005, Klugh and Cumming 2007), their responses to soil pH (Cavallazzi et al 2007), and in their colonization with plant species (Orłowska et al 2005). Therefore, selecting effective AMF strains might be an alternative choice in improving the growth of *P. mullesua* in acid soils. In this study, investigated the growth and mineral composition of *P. mullesua* plantlets to inoculation with

introduced mycorrhizal fungal inocula under the influence of an abiotic stress, the strongly acidic soil condition.

MATERIAL AND METHODS

The study was carried out in and around Doimukh area of Papum Pare district and Pasighat area of East Siang District of Arunachal Pradesh (26º30′ N-29 º30′ N Latitude and 91 º30′E-97 º30′E Longitude; altitude 100-600m asl). The region experiences a humid tropical climate (rainfall 110-160 cm; annual temperature 12 ºC-37 ºC). The vegetation type corresponds to tropical semi-evergreen forest. The soil texture of area ranges from sandy loam to loamy sand and pH ranges from 4.9-6.7.

Raising of piper plantlets: Plantlets of piper were raised through stem cuttings. The plantlets were raised in sterilized sand and soil mixture (3:1).

Isolation and collection of mycorrhizal fungi: Soil samples were collected from different locations in Arunachal Pradesh for isolation of VAM fungal spores. Samples were taken from depth of 0-15 cm under various land use systems such as forest area, jhum fields, home gardens as well as natural habitat of piper plants. Mycorrhizal fungal spores were isolated from soil . Ten AM fungal species i.e., *G. etunicatum, G. versiforme, G. albidum, G. claroidium, G.occulatum, G. macrocarpum, G. hoi, G. aggregatum, G. fasciculatum, G. aurantium* were selected to carry out the experiment.

Experimental details: A pot experiment was carried out with 11 treatments viz., non-mycorrhizal P. mullesua plantlets as control and plantlets inoculated with the above mentioned ten mycorrhizal inocula at soil pH 4.5. To maintain the required soil pH level, elemental sulfur was used (250g/10kg soil) to lower the existing soil pH up to 4.5. A healthy piper plantlet was planted in each pot inoculating with 50 gm of AM fungi cultured soil. Eight replicates of *P. mullesua* plantlet were taken per treatment. Pots were kept in mist chamber and harvesting was done after 90 days after transplanting.

Laboratory analysis: Growth parameters like shoot and root length as well as plant biomass was determined by drying them separately in hot air oven at 60 ºC for 48 hours. The percentage of the root colonized by VAM fungi were determined (Brundreett et al (1996). The chlorophyll content of leaf of *P. mullesua* was estimated by the method of Witham et al (1971). The total nitrogen and phosphorus content of plant material was determined by the Kjehldahl method and Vanadomolybdate method respectively (Juo 1982). The activity of Phosphatase was estimated by method suggested by Tabatabai and Bremner (1969).

RESULTS AND DISCUSSION

Shoot and root length: The plant growth responses with respect to plant biomass, phosphatase activity, plant nitrogen content and plant phosphorus content were significantly higher in all the plants inoculated with mycorrhizal inocula than the non-inoculated plantlets. Difference was observed in shoot length and root length among the plantlets of *P. mullesua* infected with different mycorrhizal isolates (Table 1). The plantlets of *P. mullesua* inoculated with mycorrhizal fungi *G. macrocarpum* produced highest shoot length (4.96 cm) and lowest in the seedlings inoculated with *G. aurantium* (2.833 cm) which is higher than the non-inoculated seedlings of *P. mullesua* (2.00 cm). Highest root length was in the seedlings inoculated with *G. albidum* (22.83 cm) followed by *G. aurantium* . The, lowest root length was in the seedlings inoculated with *G. aggregatum* (20.16 cm). The root length of non-mycorrhizal plantletes of *P. mullesua* was significantly lower (17.00 cm) than the plantlets inoculated with mycorrhizal fungi. Huang et al (2017) observed that in acidic soil condition plant height, branching number, shoot and root weight, and root growth all reduced with increased soil Al3+ concentrations.

Total biomass: The biomass production of *P. mullesua* plantlets varied significantly among the plantlets infected with different mycorrhizal isolates. The plantlets inoculated with *G. macrocarpum* and *G. aggregatum* (0.395 gm) showed higher biomass followed by *G. hoi* and *G. fasciculatum* (Table 1). Plantlets of *P. mullesua* inoculated with *G. versiforme* and *G. claroidium* produced least biomass (0.28 gm). The total biomass in case of non-mycorrhizal plantlets was 0.252 gm, which was less than the inoculated with mycorrhizal fungi. Shoot length and plant biomass of AM infected plants were higher than those of non-mycorrhizal controls, which confirms the results of Colla et al (2008) and Wan et al (2008).

Table 1. Shoot and root length of *Piper mullesua* seedlings inoculated with various mycorrhizal isolates at pH 4.5

VAM fungal species	Shoot length (cm)	Root length (cm)	Biomass (gm)
Control (NM)	2.00	17.00	0.252
Glomus etinucatum	3.16	20.66	0.286
G. versiforme	3.00	20.5	0.281
G. albidum	4.00	22.83	0.338
G. claroidium	3.33	20.67	0.281
G. occultum	4.66	21.67	0.371
G. macrocarpum	4.96	22.00	0.395
G. hoi	4.66	21.16	0.384
G. aggregatum	3.67	20.16	0.352
G. fasciculatum	3.16	21.33	0.311
G. aurantium	2.83	22.50	0.303

Chlorophyll content, percent infection and survivility: The highest chlorophyll content was in the seedlings inoculated with *G. hoi* (1.753 mg/gm) followed by *G. macrocarpum* and *G. aggregatum* (Table 2). Least chlorophyll content was observed in the seedlings inoculated with *G. etinucatum* (1.510 mg/gm) and it was higher than the controlled. Chlorophyll concentration was higher in the AM inoculated seedlings as compared to the non-mycorrhizal control plants, suggesting that the mineral acquisition was higher in AM infected seedlings. Wang et al (2008) observed that chlorophyll content was higher in AM plants than in uninoculated plants. Roots of inoculated *Piper mullesua* seedlings were well colonized with AM fungi and no infection was observed in uninoculated seedlings. The percentage of mycorrhizal infection in the roots of *P. mullesua* showed variation among different *Glomus* species and was highest in the roots of seedlings inoculated with *G. aurantium* (66.67%) and least with *G. fasciculatum* (23.33%) (Table 2). There was no correlation between percent root infection and pH of soil. The percentage of survivility shown highest in the seedlings inoculated with *G. aggregatum* and *G. albidum* (60%) as compared to the non-mycorrhizal plant (30%) (Table 2).

Phosphatase, phosphorus and nitrogen content of plantlets: The phosphatase content was also significantly varied among the different mycorrhizal strains and was highest in the seedlings inoculated with G. *macrocarpum* (24.9 µg/gm) followed by *G. hoi* and G. *accultum* (Table 3). The least phosphatase content was in the seedlings inoculated with *G. versiforme* (19.97 µg/gm), which is higher than the non-mycorrhizal *Piper mullesua* seedlings (17.77 µg/gm). The phosphatase content in the seedlings of both AM inoculated and non-mycorrhizal one was greater under acidic soil condition. This may be due to the fact that the extracellular and intracellular phosphatase had greater activity at low pH (Joner et al 2000) and increased in mycorrhizal colonization under this stressed condition. In the present study, the phosphatase activity showed significant positive correlation with the plant phosphorus (r=0.842). Similar result was reported by Aarle et al (2002) under P-deprived condition sand phosphatase was correlated to shoot phosphorus concentration and plant growth.

The phosphorus content in the shoots of *P. mullesua* seedlings inoculated with *G. macrocarpum* was highest (0.0255 gm $Kg^{-1}$and. least in the seedlings inoculated with *G. etinucatum* (0.0204gm Kg^{-1}) (Table 3). However the variation was not significant among different mycorrhizal isolates and non mycorrhizal one. Our results are in accordance with the previous findings. Several studies were demonstrated that edaphic conditions of acidic soils limit plant growth, mainly due to Al3+ phytotoxicity, which reduces water and nutrient acquisition from soils and severely limits root growth of sensitive species. Increased concentration of Al^{3+} cause to the damage of the root tip, leading to the inhibition of root growth and ultimately limiting the plants from adsorbing nutrients and water from soil solution (Langer et al 2009, Huang et al 2017). In such conditions, the association of symbiotic arbuscular mycorrhizal (AM) fungi with plant roots often modifies plant response to acid soil factors through enhanced P acquisition and reduced Al exposure (Aguilera et al 2015). Similar results were in present experiment where acquisition of P was higher in mycorrhizal seedlings than the non-mycorrhizal control plants . This may be due to the extending of extra radical mycelium of the mycorrhizal fungi.

Table 2. Chlorophyll, infection and survivality of *P. mullesua* seedling infected with different arbuscular mycorrhizal isolates at pH 4.5

VAM fungal species	Total chlorophyll (mg gm^{-1})	Infection (%)	Survivality (%)
Control (NM)	1.6008	-	40
Glomus etinucatum	1.7108	40.00	50
G. versiforme	1.7232	43.33	40
G. albidum	1.7368	53.33	60
G. claroidium	1.7425	46.67	40
G. occultum	1.5563	53.33	30
G. macrocarpum	1.7377	16.67	50
G. hoi	1.7530	50.00	40
G. aggregatum	1.5980	26.67	60
G. fasciculatum	1.7688	23.33	30
G. aurantium	1.7033	66.67	40

Table 3. P-ase, phosphorus and nitrogen content in the roots of *P. mullesua* seedling infected with different mycorrhizal isolates at pH 4.5

VAM Fungal species	P-ase (µg gm^{-1})	Phosphorus (gm kg^{-1})	Nitrogen (%)
Control (NM)	17.77	0.0185	0.23
Glomus etinucatum	20.13	0.0204	0.33
G. versiforme	19.87	0.0208	0.47
G. albidum	21.20	0.0214	0.47
G. claroidium	20.97	0.0216	0.37
G. occultum	23.60	0.0244	0.56
G. macrocarpum	24.90	0.0255	0.56
G. hoi	24.13	0.0231	0.51
G. aggregatum	20.40	0.0225	0.28
G. fasciculatum	23.47	0.0210	0.47
G. aurantium	21.43	0.0217	0.33

Table 4. Linear correlation coefficients between plant biomass and phosphatase (P-ase), phosphorus (P) and nitrogen (N) in *P. mullesua* seedlings infected with different mycorrhizal isolates

Mycorrhizal isolates	P-ase	P	N
Control	-0.76	0.956*	0.733
G. etinucatum	-0.439	0.53	0.122
G. versiforme	-0.392	0.152	0.6
G. albidum	-0.838	-0.6	0.452
G. claroidium	-0.318	0.766	0.980**
G. occultum	0.202	-0.979*	0.746
G. macrocarpum	0.983**	0.603	0.930*
G. hoi	-0.285	-0.526	0.045
G. aggregatum	0.164	-0.999***	-0.987**
G. fasciculatum	0.489	0.846	0.912*
G. aurantium	0.979*	0.979*	0.316

*p>0.05, **p>0.01, ***p>0.001

The effect of pH on the Nitrogen content of mycorrhizal and non-mycorrhizal seedlings was not significant (Table 3). However, nitrogen percentage was highest in *G. accultum* and *G. macrocarpum* (0.56%) whereas was only 0.23% in the shoots of non mycorrhizal seedlings. In this experiment, concentration of phosphorus and nitrogen were higher in AM infected plant than non-mycorrhizal control plants. An increase in mineral uptake by mycorrhizal plants in various plants has been reported by various workers, but the extent of increase in uptake of each element differs depending on the plant species and experimental conditions (Gaur & Adholeya 2002). Increased growth associated with AM infection in nutrient deficient soils has been attributed to enhanced nutrient uptake, especially N and P. AM infected plants appeared efficient in improving nutrient uptake, particularly in seedlings inoculated with G. macrocarpum. Similar results were recorded by Caravaca et al (2006). *G. mosseae* was the best in uptaking both the element from soil.

The correlation coefficient of plant biomass with plant phosphatase showed significant positive correlation by the seedlings infected by G. macrocarpum and *G. aurantium*. The plant phosphorus however has significant negative correlation in the seedlings infected with *G. occultum* and *G. aggregatum*. In case of non-mycorrhizal one, a significant positive correlation was observed. The positive correlation was observed in the mycorrhizal seedlings between plant biomass and plant nitrogen (Table 4).

CONCLUSION

Inoculation of mycorrhizal fungi in *Piper mullesua* plantlets resulted better plant parameters as compared to non-mycorrhizal plantlets. Among the mycorrhizal inoculums, *G. macrocarpum* and *G. hoi* were most efficient in pH 4a.5 and encouraged the growth of the plantlets. The plant biomass and phosphatase activity of *P. mullesua* plantlets were significantly increased in acidic soil on inoculation of *G. macrocarpum* fungal inocula.

REFERENCES

Aarle IM, Olsson PA and Soderstrom B 2002. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytologist* **155**(1): 173-182.

Aguilera P, Cumming J, Oehl F, Cornejo P and Borie F 2015. Diversity of arbuscular mycorrhizal fungi in acidic soils and their contribution to aluminum phytotoxicity alleviation. Pp 203-228 In: Panda SK and Balus˘ka F (eds.), *Aluminum Stress Adaptation in Plants, Signaling and Communication in Plants 24*, DOI 10.1007/978-3-319-19968-9_11.

Bala S and Singh OS 1983. Endo mycorrhizal associations of legumes and pulses in Punjab India. *Indian Journal of Ecology* **10**(2): 242-247.

Barea JM, Pozo M, L_opez-Ra´ez JM, Aroca R, Ruı´z-Lozano J M, Ferrol N, Azc_on R and Azc_on- Aguilar C 2013. Arbuscular Mycorrhizas and their significance in promoting soil-plant systems sustainability against environmental stresses. pp 353–387 In: Rodelas B and Gonzalez-Lopez J (eds) *Beneficial plant-microbial interactions: ecology and applications.* CRC Press, Boca Raton, FL.

Borie F, Rubio R, Morales A, Curaqueo G and Cornejo P 2010. Arbuscular mycorrhizae in agricultural and forest ecosystem in Chile. *Journal of Soil Science and Plant Nutrition* **10**: 185-206.

Brundrett M, Bougher N, Dell B, Grove T and Malajczuk N 1996, *Working with mycorrhizas in forestry and agriculture.* pp184-193 In: ACIAR monograph 32. Australian Centre for International Agricultural Researc. Canberra.

Caravaca F, Tortosa G, Carrasco L, Cegarra J and Roldan A 2006. Interaction between AM fungi and a liquid organic amendment with respect to enhancement of the performance of the leguminous shrub *Retama spherocarpa. Biology and Fertility of Soil* 43: 30-38.

Cavallazzy JRP, Klauberg Filho O, Stu¨rmer SL, Rygiewicz PT and Mendonc¸a MM 2007 Screening and selecting arbuscular mycorrhizal fungi for inoculating micropropagated apple rootstocks in acid soils. *Plant Cell, Tissue and Organ Culture* **90**: 117-129.

Clark RB and Zeto SK 2000. Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition* **23**(7): 867-902.

Clark RB 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant and Soil* **192**: 15–22.

Colla G, Rouphael Y, Cardarelli M, Tullio M, Rivera CM and Rea E 2008. Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biology and Fertility of Soils* **44**(3): 501-509.

Faber BA, Zasoske RJ, Munns DN and Shackel K 1991. A method for measuring hyphal nutrition and water uptake in mycorrhizal plants. *Canadian Journal of Botany* **69**(1): 87-94.

Fritz O, Endre L, Arno B, Kari S, Robert B, Marcelvander H and Ewald S 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry* **42**(5): 724- 738.

Gaur A and Adholeya A 2002. Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculums production in marginal soil amended with organic matter. *Biology and Fertility of Soil* **35**(3): 214-218.

Gerdmann JW and Nicholson TH 1963. Spores of mycorrhizal

endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**(2): 235-244.

Guo YJ, Ni Y, Raman H, Wilson BAL, Ash GJ, Wang AS and Li GD 2012. Arbuscular mycorrhizal fungal diversity in perennial pastures; responses to long-term lime application. *Plant and Soil* **351**: 389-403.

Huang L, He Y and Guo Y 2017. The efficiency of Arbuscular Mycorrhizal Fungi in promoting Alfalfa growth in Acid soil. *Journal of Agricultural Science* **9**(4): 186-204.

Ingrid M, Aarle V, Rouhier H and Saito M 2002. Phosphatase activities of arbuscular mycorrhizal intraradical and extraradical mycelium, and their relation to phosphorus availability. *Mycological Research*. **106**(10): 1224-1224.

Joner EJ and Johansen A 2000. Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. *Mycological Research* **104**(1): 81-86.

Juo ASR 1982, *Automated and semi-automated methods for soil and plant analysis manual series*, pp 33 No 7. Published and printed by International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Kelly CN, Morton JB and Cumming JR 2005. Variation in aluminum resistance among arbuscular mycorrhizal fungi. *Mycorrhiza* **15**: 193-201.

Klugh KR and Cumming JR 2007. Variations in organic acid exudation and aluminum resistance among arbuscular mycorrhizal species colonizing Liriodendron tulipifera. *Tree Physiology* **27**(8): 1103-1112.

Kochian LV, Piñeros MA and Hoekenga OA 2004. *The physiology, genetics and molecular biology of plant aluminum resistance and toxicity:* Springer Netherlands pp 175-195.

Langer H, Cea M, Curaqueo G and Borie F 2009. Influence of aluminum on the growth and organic acid exudation in alfalfa cultivars grown in nutrient solution. *Journal of Plant Nutrition* **32**(4): 618-628.

Received 22 October, 2021; Accepted 21 December, 2021

Liu RJ and LiXL 2000. *Arbuscular mycorrhiza and its application*. Science Press, Beijing. p-224.

Martens DA 2001. Nitrogen cycling under different soil management systems. *Advances in Agronomy* **70**: 143-192.

Orłowska E, Ryszka P, Jurkiewicz A and Turnau K 2005. Effectiveness of arbuscular mycorrhizal (AMF) strains in mycorrhizal colonisation of plants involved in phytostabilisation of zinc wastes. *Geoderma* **129**(1): 92-98.

Rouphael Y, Cardarelli M and Colla G 2015. Role of arbuscular mycorrhizal fungi in alleviating the adverse effects of acidity and aluminium toxicity in zucchini squash. *Scientia Horticulturae* **188**: 97-105.

Seguel A, Cumming J, Klugh-Stewart K, Cornejo P and Borie F 2013. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: A review. *Mycorrhiza* **23**: 167-183.

Smith S and Read D 2008. *Mycorrhizal symbiosis*. Elsevier, New York, NY.

Srivastva S, Gupta MM, Verma RK and Kumar S 2001. Determination of 1,3-Benzodioxanes in *Piper mullesua* by high-performance thin-layer chromatography. *Pharmaceutical Biology* **83**(6): 1484-1488.

Tabatabai MA and Bremner JM 1969. Use of p-nitrophenylphosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**(4): 301-307.

Vandamme E, Renkens M, Pypers P, Smolders E, Vanlauwe B and Merckx R 2013. Root hairs explain P uptake efficiency of soybean genotypes grown in a P-deficient Ferralsol. *Plant and Soil* **369**: 269-282.

Wang M, Christie P, Xiao Z, Qin C, Wang P, Liu J, Xie Y and Xia R 2008. Arbuscular mycorrhizal enhancement of iron concentration by *Poncirus trifoliata*.L Raf and *Citrus reticulate* Blanco grown on sand medium under different pH. *Biology and Fertility of Soils* 45(1): 65-72.

Witham FH, Blaydes DF and Devlin RM 1971, *Experiments in Plant physiology*. Van Nostrend Reinhold Company, New York.