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Full Length Research Paper

The effects of zinc (Zn) and C^{14}-indoleacetic acid (IAA) on leaf senescence ın *Helıanthus annuus* L.

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Sequential leaf senescence is defined as a kind of programmed death events which is an important process in growth of plant. This study aimed to explore the sequential leaf senescence rate due to indoleacetic acid and lack of zinc (-Zn). Therefore, the effect of zinc and indole-3-acetic acid on senescence which occurs in *Helianthus annuus* (sunflower) cotyledons was analyzed. It was found that in cotyledons of seedlings grown in Hoagland solution which was prepared without addition of zinc senescence is delayed. It was recognised that in case of ^{14}C indoleacetic acid (IAA) which was given from apical tip not reaching the root and cotyledons, senescence does not occur in cotyledons. It was studied to get more information about physiological system of sequential leaf senescence.

Key words: Sequential leaf senescence, cotyledon, zinc, ^{14}C indole-acetic acid (IAA), *Helianthus annuus* (sunflower).

INTRODUCTION

Senescence is the final phase of plant vegetative and reproductive development, preceding the widespread death of cells and organs (Schmid et al., 1999; Guiboileau et al., 2010; Caswell and Salguero-Gomez, 2013). It has long been known that hormones regulate the progression of leaf senescence (Fletcher and Osborne, 1965; Misra and Biswal, 1980; Noodén and Leopold, 1988; Jibran et al., 2013). In the process of senescence, destruction cases occur more than synthesis. From point of that view, definition of senescence is the process which increases destruction cases in cell and causes the plant to die.

The analysis made on leaf cells shows that during senescence consecutive metabolic events occur. These events can be ordered as the synthesis of proteolitic enzyme (Colin and Thimann, 1972; Cheng and Kao, 1984; Hörtensteiner and Feller, 2002), the start of destruction of membrane proteins caused by these enzyme's activities, the decrease of quantity of protein and total nitrogen in the cell (Krul, 1974; Peterson and Huffaker, 1975; Peoples and Dalling, 1978, Prakash et al., 2001; Hopkins et al., 2007; Kaplan-Dalyan and Sağlam-Çağ, 2013), the acceleration of chlorophyll destruction (Peoples et al., 1980; Rodoni et al., 1997; Hörtensteiner, 2006; Darnel et al., 1990) and lipid destruction (Dhindsa et al., 1982; Harwood et al., 1982; Thompson et al., 1998; Hebeler et al., 2008). It is accepted that transportation of nutrients in other leaves

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starting from the oldest organ to the youngest supports the nutrient drain hypothesis. According to another hypothesis called 'signal' hypothesis, a signal which is thought to be synthesized by developing seeds, is being transported to old leaves and causes senescence as a result of catabolic reactions. According to this hypothesis, if the signal center is eliminated, senescence does not occur (Lindoo and Noodén, 1977). But the above mentioned signal has been displayed that it could not be isolated (Moore, 1979; Ridge, 1991). It is obviously known that the cause of all biochemical events during senescence are releated to gene expression (Draper, 1969; Sanders and Write, 1995; Hörtensteiner, 1997; Distelfeld et al., 2014). The meanings of these chemical events come into being only with the researches made by plant physiologs on plant's physiology. While it is being said that auxins (Wareing and Seth, 1967; Kahanak et al., 1978; Lim et al. 2003) delay senescence, researches made recently indicate that auxins (Palni et al., 1988; Lu et al., 2001; Gören and Sağlam-Çağ, 2007) accelerate the senescence. Otherwise, Noh and Amasino (Noh and Amasino, 1999) detected that auxin represses transcription of some genes whose expression is correlated with senescence.

MATERIALS AND METHODS

Helianthus annuus L. seedlings were grown in intensity of 6000 lux light, under 12 h photoperiod and 26 ± 2°C.

Designation of senescence degree

To determine the senescence which occur in cotyledons of *H. annuus* quantitatively the method improved and used for soya bean's Anoca variety-show by Lindoo and Noodén (1976) was adapted to *H. annuus* cotyledons. For chlorophyll designation Arnon (1949) method was used. To determine total nitrogen quantity a method, formed with combination of Kjeldahl method and spectrophometric measurement method was used (Lindoo and Noodén, 1976). The zinc quantity in the material was designated with atomic absorption spectrophotometer (AAS).

Giving IAA to the cut ends of the decapitated seedlings

One to two days before senescence starts in cotyledons, seedlings were decapitated by being cut approximately 3 cm above the internodium cotyledons. 10^{-5} M. IAA solution (treated) or water (control) was applied to decapitated surface.

Giving ^{14}C–IAA to the seedlings on the top buds and nodium leaves

10^{-5} mol. ^{14}C-IAA (specific activity:40 mCi / mmol.) 1 drop 1% tween-20 was added per 1 ml. 60 and 120 µl from IAA solution was dropped on plant's top bud, 80 and 320 □ lwas dropped on nodium leaves. To hinder indolacedic acid's photo oxidation, this process was made when the plants were passing to dark period. The whole organ, of which radioactivity will be enumerated in β counter was prepared accordingly. Counting value of the material per 5 min (cp5m=count per 5 min) was calculated.

Statistic evaluation of the results

Standard deviation estimate was made to evaluate the results obtained from experiment and control groups statistically. In case of the number obtained when ± values of differences between the result's square's total sum's square root is multiplied with three is found to be smaller than the difference between values, the difference is decided to be significant statistically.

RESULTS

As it is known that zinc provides indole-acetic acid (IAA) stabilization and in case of zinc deficiency quantity of IAA decreases, this mineral's effects on plant growing and cotyledon senescence were analysed. The seedlings forming the experiment group were grown in Hoagland solution which does not include zinc and was diluted in 1/8 ratio (Table 1). Senescence delayed in cotyledons of seedlings in -Zn solution. Besides with the ingathering, when cotyledon senescence in plants grown with the existence of zinc (control) is 50% according to plastochron index (28th day), total chlorophyll and total nitrogen quantities in cotyledons of all seedlings belonging to experiment or control group (Table 2). The quantity of the zinc which is thought to exist in the seed naturally was measured with AAS (Figure 1). Nineteen days old *H. annuus* seedlings were devided into 4 groups. First group plants were intact (control). Other group plants were given IAA, NAA and H_2O from truncated end being decapitated from under 2nd internodium. After this process the speed of senescence occuring in cotyledons was observed (Figure 2). In cotyledons of plants to which IAA and NAA applied senescence occured quickly just like it occurs in cotyledons of intact plants. However, in a great majority of plants having a process with H_2O, cotyledons remained green. Senescence did not occur in the cotyledons of the 17 days old experiment and control seedlings which were exposed to the same process and application. To determine first which organ as a target indolacedic acid after being produced in the plant is transported, 120 µl from 10^{-5}M ^{14}C-IAA+tween 20 upon top bud of the plants was dropped in. After dark period for 12 h, the quantity of ^{14}C-IAA in the roots and cotyledons of the plants was stated (Table 3). Radioactivity existing in the root is found to be more than 20 times more than the radioactivity in cotyledons. From the values obtained it was understood that IAA given from top bud was transported quickly to the root. On the other hand, from the middle of the first internodium's of the seedlings, a part, approximately 1 cm was boiled with hot water vapour on the 17 day 60 µl 10^{-5} M-IAA+tween 20 was dropped in the top bud of the plants of which cotyledons was just 100% gren on the 32 day. After dark period for

Table 1. The effect of Zn (105 mg/L) on the green area (%) of the cotyledons from-day *H. annuus* seedlings grown in 1/8 Hoagland solution.

Day	Average green area Percentage [Control (+ Zn)]	Day	Average green area Percentage [Control (- Zn)]
17	100.00 ± 0.00	20	100.00 ± 0.00
22	78.00 ± 0.26	23	73.93 ± 1.62
25	56.34 ± 1.42	27	49.45 ± 1.23
29	32.73 ± 2.46	30	21.61 ± 1.72
33	00.00 ± 0.00	34	00.00 ± 0.00

Table 2. The effect of Zn (105 mg/L) on the chlorophyll and nitrogen content of the cotyledons from 28 day *H. annuus* seedlings grown in 1/8 Hoagland solution.

Hoagland	mg N / g cotyledon	%	mg chlorophyll / cotyledon	%
Hoagland (- Zn)	129.603 ± 4.256	100	0.0449 ± 0.005	100
Hoagland (+ Zn)	108.109 ± 2.562	83.4	0.0382 ± 0.002	74.2

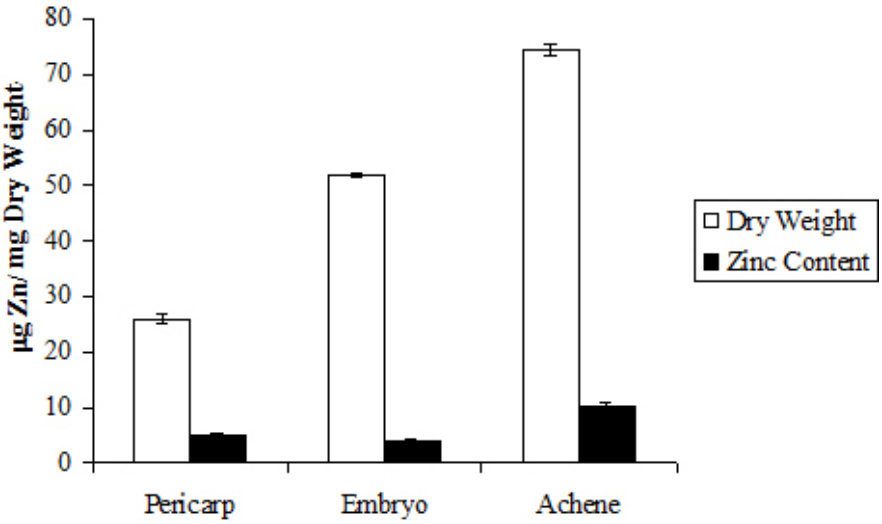

Figure 1. Dry weight and Zn quantity in pericarp, embryo and achene.

12 h, radioactivity in different organs of the plants was measured (Table 4).

As the radioactivity difference counted in organs under the 1 internodium's boiled part was unsignificant statistically, it was understood that radioactivity can pass downwards from boiled area in trace quantity. Besides, althought it was found on boiled part, on the leaves of the 2 internodium a statistically significant quantity of radioactivity could not be found. It was stated that radioactivity had accumulated in a great quantity on the boiled part of internodium.

To the first leaves (second nodium leaf) after cotyledon of 19 days old plants totally 80µl ^{14}C-IAA, 40 per each, was applied. After plants being ingathered on different days (Avery et al., 1937; Lindoo and Noodén, 1976; Papadopoulus et al., 1985; Noodén and Leopold, 1988), radioactivity on root and 3. nodium leaves was measured (Table 5).

It was recognised that ^{14}C-IAA applied to 2 nodium leaves was transported to root first and then from there by xylem, was transported to the leaves making transpiration.

DISCUSSION

In this research, the effects of zinc and indolacedic acid, which is a growing hormone, on senescence was

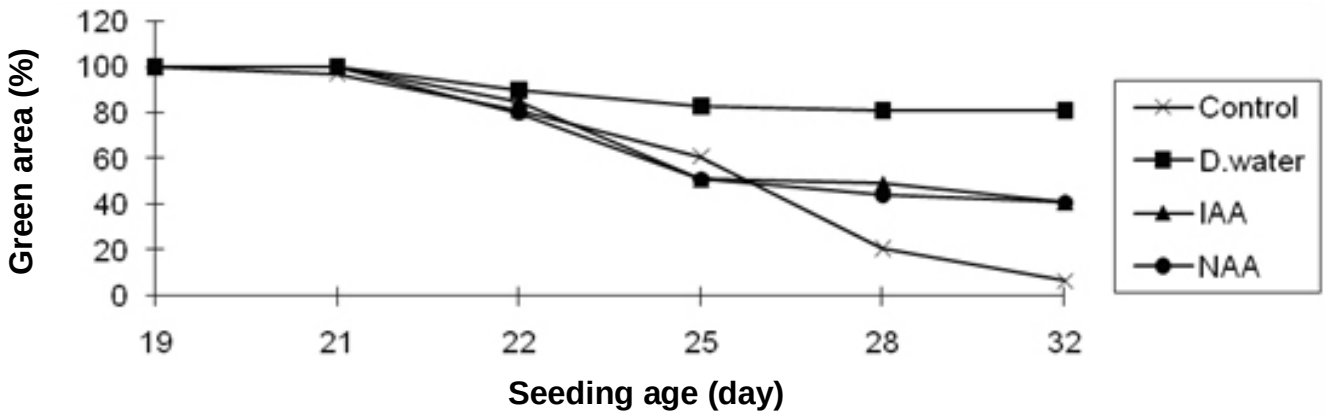

Figure 2. Green area (%) of cotyledons of 19 days old *H. annuus* seedlings which were decapitated below the second internode and treated with 10^{-5}M IAA, 10^{-5}M NAA or H_2O from the truncated end.

Table 3. ^{14}C amounts in the roots and cotyledons of *H. annuus* seedlings treated with 10^{-5} M ^{14}C-IAA (* Significant).

Organ	Count / 5 min	Radiation of background	Difference
Cotyledon	299.85 ± 31.25	288.36 ± 3.015	11.49
Root	875.41 ± 29.09	288.36 ± 3.015	587.05 *

Table 4. ^{14}C amounts in different organs of *H. annuus* seedlings treated with 10^{-5} M ^{14}C-IAA (* Significant) .

Organ	Count / 5 min	Radiation of background	Difference
2nd node leaves	306.92 ± 7.05	288.36 ± 3.015	18.56
First internode	1039.33 ± 57.63	288.36 ± 3.015	750.97 *
100% Green cotyledon	299.87 ± 7.66	288.36 ± 3.015	11.51
Hypocotyl	304.00 ± 11.82	288.36 ± 3.015	15.64
Root	313.00 ± 10.64	288.36 ± 3.015	24.64

Table 5. ^{14}C amounts in the roots and 3rd node leaf of *H. annuus* seedlings leaf on different days after 10^{-5} M ^{14}C-IAA treatments on the 2nd nodium. (* Significant).

Day	Root (Count / 5 min)	3rd nodium leaf (count / 5 min)	Radiation of background	Difference (root)	Difference (3 rd nodium leaf)
20	318,44 ± 11.627	302,67 ± 3.567	288.36 ± 3.015	30.08	14.31 *
21	489,11 ± 34.310	352,22 ± 10.97	288.36 ± 3.015	200.75*	63.86 *
22	454.00 ± 45.287	342.00 ± 14.09	288.36 ± 3.015	165.64 *	53.64 *
25	485,67 ±11.794	712,89 ± 131.27	288.36 ± 3.015	197.31 *	424.53 *

searched. In a reseach (Ray and Choudhuri, 1981), it was supported that hormones (IAA, GA, Kinetin) plays the most important role in transporting nutrients to seeds that develops as an endogenic hormone resource. It is known that the deficiency of the hormones which perevents senescence (for ex: cytokinin) may cause senescence. Palni et al. (1988) mentions that auxin has an effect on cytokinin's metabolism and this effect is actualized by oxidase enzyme. While some researchers (Jacobs and Cready, 1967; Sanchez-Bravo et al., 1991) declare that indolacedic acid localize in cortex, vascular tissue and pith, auxin is transported in vascular and epidermal tissues, other researchers (Bangerth, 1994; Ekölf et al., 1995; Li et al., 1995; Shimizu-Sato et al., 2009)

emphasized that intact plants's cytokinins in xylem exudate are under the control of polar auxin transportation system. Hare and Staden (1994) expressed that cytokinin catabolism which becomes true with the activity of sitokinin, a specific enzyme oksidase realizes death in plant tissue and moreover stated that auxin plays the role of allosteric systematizer increasing this enzyme's activity.

It is known that indolacedic acid is synthesized in the end of stem and zinc provides the stabilization of indolacedic acid (Skoog, 1940; Takaki and Kushizaki, 1970). In the early development phase of the plants of which endogen IAA quantity was decreased by being grown in zinc deficiency, IAA that is under the control of the quantity of zinc in the seed has such a quantity that it delays the senescence but can provide growing. But zinc which is given with Hoagland solution in addition to the zinc quantity in the seedling may be impulsive in senescence or may delay growing because of its toxical effects on some enzyme systems releated to growing. Likewise, Sağlam-Çağ and others (Sağlam-Çağ et al., 2004) emphasized that senescence was delayed in excised cotyledons in the solution lacking zinc. In that research, in the existence or deficiency of zinc, IAA which can be controlled endogenly was hold to be responsible for the change in senescence's speed. In some experiments which ^{14}C-IAA was used (Hew et al., 1967), it was noticed that IAA given from truncated end of the stem goes through stem axis quickly and don't enter to leaves. Also, in this research it was found that ^{14}C-IAA was transported to root without touching at leaves. Moreover, as ^{14}C's internodium does not goes through boiled part, it was noticed that it could not reach the root and cotyledons and senescence didn't occur in cotyledons.

We can assert that sequential leaf senescence is releated to the occurance of metaxylem after protoxylem and in this event, with IAA's effect on xylem formation, it may come on the scene. Just before senescence, although senescence occured when IAA was applied from truncated top, cotyledons remained green when IAA was applied in early phase. Researhers (Shimomura et al., 1988; Jones et al., 1989; Jones, 1994) indicated that there are 2 different receptor in plasma membrane connecting IAA and in recent years it was determined that first one of these receptors isolated is releated to cell growing but then any absolute information about second IAA receptor's function wasn't given (Darnel et al., 1990; Cooper, 1997).

As a result of our research, we saw that zinc, providing IAA stabilization accelerates senescence; in the researches made with ^{14}C, as its internodium does not go through boiled part ^{14}C-IAA given from apex, it can not reach root and cotyledons and senescence does not occur in cotyledons. It became certain that it was transported to the root without touching at leaves and this transportation is made by parenchymatic living tissues not xylem. This research indicated that senescence signal may be indole-acetic acid or a substance like indoleacetic acid.

Conflict of interests

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

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REFERENCES

Arnon DI (1949). Copper enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris.* Plant Physiol. 24:1-15.

Avery GS, Burkholder Jr. PR, Creighton HB (1937). Nutrient deficiencies and growth hormone concentration in *Helianthus* and *Nicotiana.* Am. J. Bot. 24:553-57.

Bangerth F (1994). Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus vulgaris* L.) plants to decapitation and auxin treatment, and relationship to apical dominance. Planta 194:439-442.

Caswell H, Salguero-Gomez R (2013). Special feature new perspectives in whole-plant senescence: Age, stage and senescence in plants. J. Ecol. 101:585-595.

Cheng SH, Kao CH (1984). The role of proteolytic enzymes in protein degradation during senescence of rice leaves. Physiol. Plantarum 62:231-237.

Colin M, Thimann K (1972). Role of protein synthesis in the senescence of leaves. Plant Physiol. 50:432-437.

Cooper GM (1997). The cell: A molecular approach. ASM Press. Washington, D.C. Sinauer Associates, Inc.Sunderland, Massachusetts. pp. 521-560.

Darnel J, Lodish H, Baltimore D (1990). Molecular Cell Biology. Second Ed. Scientific American Books. W.H. Freeman Company, Newyork. p. 709-762.

Dhindsa RS, Plumb-Dhindsa PL, Reid DM (1982). Leaf senescence and lipid peroxidation: Effects of some phytohormones, and scavengers of free radicals and singlet oxygen. Faculdade de Agronomia, Univ. Eduardo Mondlane, Maputo, Mozambique, Department of Biology, University of Calgary, Calgary, Alberta T2N 1N4, Canada.

Distelfeld A, Avni R, Fischer AM (2014). Senescence, nutrient remobilization, and yield in wheat and barley. Jour. Experiment. Bot. 27:1-16.

Draper SR (1969). Lipid Changes in Senescing Cucumber Cotyledons. Phytochemistry 8:1641-1647.

Ekölf S, Astot C, Blackwell J, Moritz T, Olsson O, Sandberg G **(**1995). Auxin/cytokinin interactions in wild-type and transgenic tobacco. Plant Cell Physiol. 38:225-235.

Fletcher RA, Osborne DJ (1965). Regulation of protein and nucleic acid synthesis by gibberellin during leaf senescence. Nature 207:1176-1177.

Gören N, Sağlam-Çağ S (2007). The Effect of Indole-3-acetic acid and benzyladenine on sequential leaf senescence on *Helianthus annuus* L. seedlings. Biotechnol. Biotec. Eq. 21:322-327.

Guiboileau A, Sormani R, Meyer C, Masclaux-Daubresse C (2010). Senescence and death of plant organs: nutrient recycling and developmental regulation. CR. Biol. 333:382-91.

Hare PD, Van Staden J (1994). Cytokinin Oxidase: Biochemical features and physiological significanse. Physiol. Plant., 91:128-136.

Harwood JL, Jones AVHM, Thomas H (1982). Leaf Senescence in a non-yellowing mutant of *Festuca pratensis.* III. Total acyllipids of leaf tissue during senescence. Planta. 156:152-157.

Hebeler R, Oeljeaklaus S, Reidegeld KA, Eisenacher M, Stephan C, Warscheid B (2008). Study of early leaf senescence in *Arabidopsis thaliana* by quantitative proteomics using reciprocal 14N/15N labeling and difference gel electrophoresis. Mol. Cell Proteomics 7:108-20.

Hew CS, Nelson CD, Krotkow G (1967). Hormonal kontrol of translocation photosynthetically assimilated ^{14}C in young soybean plants. Am. J. Bot. 54:252-256.

Hopkins M, Taylor C, Liu Z, Ma F, McNamara L, Wang T, Thompson JE (2007). Regulation and execution of molecular disassembly and catabolism during senescence. New Phytol. 175:201-214.
Hörtensteiner S (1997). Chlorophyll breakdown in senescent chloroplasts (cleavage of pheophorbide a in two enzymic steps). Plant Physiol. 115:669-676.
Hörtensteiner S (2006). Chlorophyll degradation during senescence. Annu. Rev. Plant Biol. 57:55-77.
Hörtensteiner S, Feller U (2002). Nitrogen metabolism and remobilization during senescence. J. Exp. Bot. 53:927-937.
Jacobs WP, Mc Cready CC (1967). Polar transport of growth-regulators in pith and vascular tissues of coleus stems. Am. J. Bot. 54:1035-1040.
Jibran R, Hunter DA, Dijkwel PP (2013). Hormonal regulation of leaf senescence through integration of developmental and stress signals. Plant Mol. Biol. 82:547-561.
Jones AM (1994). Auxin-binding Proteins. Annu. Rev. Plant Phys. 45:393-420.
Jones AM, Lamerson P, Venis MA (1989). Comparison of Site I Auxin-binding and a 22-kilodalton Auxin-binding Protein Maize. Planta 179:409-413.
Kahanak GM, Okatan Y, Rupp DC, Noodén LD (1978). Hormonal and genetic alteration of monocarpic senescence in soybeans. Plant Physiol. 61:26.
Kaplan-Dalyan E, Sağlam-Çağ S (2013). The effect of epibrassinolide on senescence in horizontal sunflower (*Helianthus annuus* L.) seedlings. IUFS J Biol, 72(1):33-44
Krul WR (1974). Nucleic acid and protein metabolism of senescing and regenerating soybean cotyledons. Plant Physiol. 54:36-40.
Li CJ, Guevara E, Herrera J, Bangerth F **(**1995). Effect of apex excision and replacement by 1-naphthylacetic acid on cytokinin concentration and apical dominance in pea plants. Physiol. Plantarum 94:465-469.
Lim PO, Woo HR, Nam HG (2003). Molecular genetics of leaf senescence in Arabidopsis. Trends Plant Sci. 8:272-278.
Lindoo SJ, Nooden LD (1977). Studies on the behavior of the senescence signal in *anoka soybeans.* Plant Physiol. 59:1136-1140.
Lindoo SS, Noodén LD (1976). The interrelation of fruit development and leaf senescence in anoka soybeans. Bot. Gaz. 137:218-223.
Lu IL, Sutter E, Burger D (2001). Relationships between benzyladenine uptake, endogenous free IAA levels and peroxidase activities during upright shoot induction of Cymbidium ensifoilum cv. Yuh Hwa rhizomes in vitro. Plant Growth Regul. 35:161-70.
Misra AN, Biswal UC (1980). Effect of phytohormones on chlorophyll degradation during aging of chloroplasts in vivo and in vitro. Protoplasma 105(1-2):1-8.
Moore TC (1979). Biochemistry and physiology of plant hormones. In chapter 6 Ethylene. Springer Verlag, New York. 208-229.
Noh YS, Amasino RM (1999). Identification of a promoter region responsible for the senescence-specific expression of SAG12. Plant Mol. Biol. 41:181-194.
Noodén LD, Leopold AC (1988). Senescence and aging in plants. Academic press, San Diego.
Palni LMS, Burch L, Horgan R (1988). The effect of auxin concentration on cytokinin stability and metabolism. Planta 174:231-234.
Papadopoulus I, Rending VV, Broadbent FE (1985). Growth, nutrition and water uptake of tomato plants with divided roots growing in differentially salinized soil. Agron. J. 77:21-26.
Peoples MB, Beilharz WC, Waters SP, Simpson RJ, Dalling MJ (1980). Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.). II. Choloroplast senescence and the degradation of ribulose-1,5-BiP carboxilase. Planta 149:241-251.
Peoples MB, Dalling MJ (1978). Degradation of ribulose-1,5 biphosphate carboxylase by proteolytic enzymes from crude extract of wheat leaves. Planta 138:153-160.
Peterson LW, Huffaker RC (1975). Loss of ribulose 1,5 diphosphate carboxilase and increase in proteolytic activity during senescence of detached primary barley leaves. Plant Physiol. 55:1009-1015.
Prakash JSS, Baig MA, Mohanty P (2001). Senescence induced structural reorganization of thylakoid membranes in Cucumis sativus cotyledons; LHC II involvement in reorganization of thylakoid membranes. Photosynth. Res. 68:153-161.
Ray S, Choudhuri MA (1981). Effects of Plant Growth Regulators on Grain-filling and Yield of Rice. Ann. Bot.-London 47:755-758.
Ridge I (1991). Plant physiology: The regulation of plant growth. Hodder and Stoughton. The Open University. pp. 7:282-333.
Rodoni S, Muhlecker W, Anderl M, Krautler B, Moser D., Thomas H, Matile P, Hortensteiner S (1997). Chlorophyll breakdown in senescent chloroplasts (cleavage of pheophorbidea in two enzymic steps). Plant Physiol. 115:669-676.
Sağlam-Çağ S, Cevahir G, Ünal M, Kaplan E, Çıngıl Ç, Kösesakal T (2004). The effect of Zn, Cu, Mn on senescence in excised cotyledons of *Eruca sativa* L. Fresen. Environ. Bull. 13:733-739.
Sanchez-Bravo J, Ortuna A, Botia JM, Acosta M, Sabater F (1991). Lateral diffusion of polarly transported indoleacetic acid and its role in the growth of *Lupinus albus* L. hypocotyls. Planta 185:391-396.
Sanders EJ, Write MA (1995). Programmed cell death in development. Int. Rev. Cytol. 163:105-173.
Schmid M, Simpson D, Giet C (1999). Programmed cell death in castor bean endosperm is associated with the accumulation and release of a cysteine endopeptidase from ricinosomes. PNAS 96:14159-14164. Senescence and death of plant organs: nutrient recycling and developmental regulation. CR. Biol. 333:382-91.
Shimizu-Sato S, Tanaka M, Mori H (2009). Auxin-cytokinin interactions in the control of shoot branching. Plant Mol. Biol. 69:429-435.
Shimomura S, Inohara N, Fukui T, Futai M (1988). Different Properties of two Types of Auxin-binding Sites in Membranes from Maize Coleoptiles. Planta 175:558-566.
Skoog F (1940). Relationships between zinc and auxin in the growth of higher plants. Am. J. Bot. 27:939-951.
Takaki H, Kushizaki M (1970). Accumulation of free triptophan and triptamine in zinc deficient maize seedlings. Plant Cell Physiol. 11:793-804.
Thompson JE, Froese CD, Madey E, Smith MD, Hong Y (1998). Lipid metabolism during plant senescence. Prog. Lipid. Res. 37:119-141.
Wareing PF, Seth AK (1967). Ageing and senescence in the whole plant. In HW Woolhouse, ed, Aspects of the Biology of Ageing. Symposium of the Society for experimental Biology, Academic Press, New York 21:543-558.

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Interactive effects of virus and *Rhizobium* inocula on nodulation, growth and yield of cowpea

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The effect of separately inoculating cowpea cultivars, 'Ife brown" (IF) and "Owode" (OW) with *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea yellow mosaic virus* (CYMV) and *Rhizobium* IRc 284(RH-284) on nodulation was investigated. Also, the effect of inoculating the cowpea cultivars with RH-284 on the severity of infection caused by the viruses was studied. The interactive effects of inoculating cowpea with RH- 284 and each of CABMV, CYMV, *Southern bean mosaic virus* (SBMV) and *Blackeye cowpea mosaic virus* (BICMV) on nodulation, growth and yield of IF and OW were also investigated. The results showed that infection by CYMV and CABMV significantly reduced nodulation by about 20-30% and 40-45% in IF and OW, respectively. Inoculating with RH-284 alone significantly increased nodulation by about 20% in both cowpea cultivars. In the interactive study involving virus-RH 284 inocula, slight but non-significant increases of 22, 2 and 9% in nodule number were observed in IF inoculated with RH-284 and SBMV, CYMV and CABMV, respectively. The differences observed in the nodule, shoot and seed weights were not significantly different from those of the control. There was a negative correlation between nodule number and severity of symptom. BICMV caused the most severe effect on the two cowpea cultivars. It reduced the number of nodules by 55-66% with or without RH-284. It also caused significant reductions of over 80% in nodule and seed weights of OW. In conclusion, increase in nodulation reduced viral disease severity, the slight but non-significant increases observed in the growth and yield parameters suggest that improved nodulation can be advantageous to cowpea.

Key words: Blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, cowpea yellow mosaic virus, southern bean mosaic virus.

INTRODUCTION

Cowpea is an important food and fodder legume in the sub-humid tropics of Africa. As a food, the grain is an important source of dietary protein especially for the West African populace where two-thirds of the world's cowpea grain is produced. The crop has therefore attracted a lot of attention from researchers who have in recent years intensified their efforts at improving its agronomic and nutritional qualities (Rachie, 1985).

Cowpea grain yields vary greatly in different parts of the world. Singh (1980) estimated that the average yield for the crop grown in monoculture is about 1.5 t/ha in the United States of America, 650 kg/ha in South America and Asia, and is often below 400 kg/ha in Africa. The low cowpea yield in Africa is mainly due to pests and

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diseases. Among the numerous pathogens the effect of these viruses can be devastating and they are a major constraint to increased grain production. Although, nine viruses are reported on cowpeas in sub-Saharan Africa (Taiwo, 2003), only *Cowpea aphid- borne mosaic virus* (CABMV) genus *Potyvirus* and *Cowpea yellow mosaic virus* (CYMV) genus *Comovirus* are considered to be very important as far as geographical distribution, pathogenic variability and yield losses are concerned (Thottappilly and Rossel, 1992). *Cowpea mottle virus* (CMeV) genus *Carmovirus, Southern bean mosaic* (SBMV) genus *Sobemovirus*, *Cowpea golden mosaic* (CGMV) genus Begomovirus, Blackeye cowpea mosaic virus (BICMV) genus *Potyvirus* and *Cucumber mosaic* viruses (CMV) genus *Cucumovirus* are considered to be of localized importance (Taiwo, 2003).

Yield reduction attributable to CYMV infections range from 40-100% (Chant, 1960; Wells and Deba, 1961; Shoyinka, 1974; Gilmer et al., 1974) while a virus suspected to be CABMV caused a complete loss in yield in northern Nigeria (Raheja and Leleji, 1974).

Efforts to control viral diseases of cowpea may involve one or more measures intended to reduce sources of infection, roguing of diseased plants and the use of insecticides to prevent virus transmission. Currently, host plant resistance is the most effective method of controlling cowpea viruses in Africa (Thottappilly and Rossel, 1992).

However, like many other legumes, cowpea can symbiose with nodule bacteria present in most tropical soils. Effective cowpea-*Rhizobium* symbiosis fixes more than 150 kg/ha of N_2 and supply a considerable amount of the N_2 requirement of plants (Summerfield et al., 1977). This attribute allows healthy growth and hence optimum yields. Symbiotic N_2 fixation in cowpea-root nodules is a result of the complex biological and biochemical interactions between the host legume and the rhizobial endophyte. If the process is efficient, the plant grows healthily, thereby minimizing the impact of diseases. It is therefore, expected that the extraneous application of *Rhizobium* to cowpea at planting should increase its population in the soil, ensure optimal and rapid growth of the crop, and thus enhance its resistance to phytopathogenic agents, especially viruses.

This study was therefore carried out to assess the effect of rhizobial inoculant on growth and seed yield of cowpea, the impact of viral infection on nodulation, vegetative and seed yield of the crop and determine the extent of amelioration of the impact of viral infection on cowpea by rhizobial inoculant.

MATERIALS AND METHODS

Two greenhouse studies were carried out to determine the effect of inoculating 2 cowpea (*Vigna unguiculata* L. Walp) genotypes (Ife Brown (IF) and Owode (OW)) with cowpea rhizobium IRc 284 (RH-284) and virus. The interaction of the microorganisms on the 2 cowpea genotypes was also evaluated.

Preliminary greenhouse 1

It was a factorial experiment consisting of 2 cowpea genotypes (IF and OW, one rhizobial inoculant (RH-284) I+ and Io and 2 virus strains, CABMV and CYMV, and control. Thus 12 treatment combinations were obtained. The treatments were replicated 3 times and arranged in a randomized complete block design (RCBD). In this study, only nodule number was assessed.

Greenhouse 2

In this experiment, a more detailed study was carried out using the 2 genotypes in the preliminary work as well as rhizobium strain. However, additional viruses, BICMV as well as SBMV were used. Therefore, a factorial design that consisted of 2 cowpea genotypes, 2 rhizobial inoculants I+ and Io and 4 virus strains with a control was set up. The twenty treatment combinations obtained were replicated 8 times.

Rhizobial inoculation

Seeds of IF and OW were inoculated with the cowpea *Rhizobium* IRc 284 (RH-284) (obtained from the culture bank of the International Institute of Tropical Agriculture (IITA)) at a concentration of 10^7 - 10^8 colony forming unit (CFU) before planting in soil at three seeds per pot.

The seeds for the control plants were not inoculated with the rhizobial inoculum. The RH-284 inoculum was prepared in yeast extract mannitol broth (YEM) (Vincent, 1970), (Mannitol, 20.0 g; $(NH_4)_2SO_4$ 1.0g; $MgSO_4.7H_20$, 0.5g; yeast extract, 0.2 g; $FeCl_3$, 2.0 mg; $MnSO_4.H_2O$, 4.0 mg; in 1 L of distilled water pH 6.8). The medium (100 ml/flask) was autoclaved at 121°C for 15 min, cooled, inoculated with RH-284 and incubated for about 7 days on a rotary shaker at 28°C, before being used as inoculum. The number of colony forming units (CFU) on plate count agar was 10^8. Numbers of nodules on 3 plants of the replicates each of RH-284 inoculated and un-inoculated plants were determined after six weeks. A photograph of nodules on roots was also taken.

Virus inoculation

Three seeds were separately sown per pot. The seedlings were later thinned to 2. Seedlings of 8-day old plants of IF OW that were to be inoculated were mechanically inoculated with the viruses. Mechanical transmission was with viral inocula prepared in 0.01 M phosphate buffer pH 7.1 according to Nordam (1973), while buffer inoculated plants served as control. The inoculated plants were labeled and kept in an insect-proof screen house at temperatures of 28-32°C. They were regularly observed for symptom development. The severity of symptoms observed was rated on a scale of 1 - 5, (5 for very severe infection, sometime death, 4 for severe infection, 3 for moderate infection; 2 for mild infection and 1 for very mild infection).

Plants were harvested after six weeks, the plants were carefully uprooted, the soil was washed off and the nodules on the roots counted.

Nodule number and weights, vegetative weights were determined on 3 of the 8 replicated plants while seed yield was determined on the other replicates at maturity.

Analysis of variance (ANOVA) was used to determine significant differences and means of the significantly different sources were separated using Duncan multiple range test.

Table 1. Analysis of variance table for nodule number in preliminary Experiment.

Variation	df	Mean sum of square
Cowpea (block)	1	0.798
Virus	4	16.69**
Inoculant (RH)	1	39.33**
Cowpea x RH	1	0.090
Cowpea x Virus	4	0.040
Virus x RH	4	1.990
Cowpea x Virus x RH	4	0.356
Error	24	5.267

** Significant at P≤0.01.

Table 2. Effect of viral and rhizobial inoculation on nodulation in the preliminary study.

	Treatment	Nodule number
Virus inoculant treatments	control	5.83[a]
	CYMV	4.35[b]
	CABMV	3.87[b]
Rhizobia inoculant treatments	control	3.78[b]
	RH-284	5.59[a]
	Standard error	0.34

Means followed by same letter in a column in each treatment are not significantly different.

Figure 1. Effect of virus on nodulation in roots of A: healthy and B: CABMV = Cowpea aphid-borne mosaic virus-infected cowpea plants.

RESULTS

Analysis of variance in Table 1 showed that the 2 viruses had significant ($p<0.01$) effect on number of nodules in the 2 cowpea genotypes in the preliminary study. Rhizobium inoculant significantly impacted on the number of nodules. The number of nodules on Ife Brown (IF) and Owode (OW) cowpeas was not significantly different (Table 2). Cowpea genotypes without virus had higher number of nodules when compared with virus infected plant. The number of nodules was significantly higher ($p<0.01$) than that of plants inoculated with CYMV and CABMV by over 20 and 30%, respectively. Number of nodules on plant inoculated with CABMV and CYMV were not significantly different. Rhizobium significantly ($p<0.01$) increased nodule number by 30% when compared with uninoculated plant.

Also, in the preliminary study, virus infection adversely affected the number and size of the nodules in the cowpea genotypes. Fewer and smaller nodules were formed and the growths of root hairs as well as the lateral roots were impaired in virus-infected plants (Figure 1).

In the second greenhouse study, nodule numbers were not significantly different from each other in the 2 cowpea genotypes but vary significantly with respect to nodule weight in the analysis of variance (Table 3). The viruses impacted significantly on nodule number and weights. There were also significant ($p<0.01$) cowpea-virus interaction as well as virus-rhizobium interaction on nodule weight. The 3 factors also interacted on the number of nodules (Table 3).

Root weights in the 2 cowpea genotypes varied significantly from each other but not with the shoot weight (Table 4). There was significant interaction of cowpea and virus on root while virus and rhizobium interaction significantly impacted on weight of shoot. Viruses as well as rhizobium had significant effect on weight of seeds.

Table 5 shows the effect of variety, rhizobium and virus on nodulation, vegetative and seed yield of cowpea. The number of nodules in the 2 cowpea genotypes did not differ significantly but the weight of nodules, root and shoot in Ife Brown were significantly higher than that of Owode variety. Owode genotype however, gave a significantly higher seed yield than IF. Rhizobium significantly increased nodule number but not nodule weight. The nodule weights were also significantly increased by RH-284. Numbers of nodules were significantly reduced by BICMV (IT16) when compared with other viruses and control. Nodule weight in BICMV was significantly reduced by 50% relative to virus-free control. Nodule weight in BICMV-treated cowpea was 30% lower than control and SBMV-treated plants. Weight of shoot and seed were also significantly reduced by 60 and 50% respectively by BICMV relative to control. Seed weights of cowpeas inoculated with CYMV and CABMV were significantly reduced relative to control.

The interactive effect of the 3 factors, namely; variety, rhizobium, rhizobium and viruses on the parameters are

Table 3. Analysis of variance table for nodule number and weight in green house (Experiment 2).

Variation	df	Mean sum of square (number of nodule)	Mean sum of square (weight of nodule)
Cowpea (Block)	1	0.86	2.32**
V	4	129.84**	9.81**
Innoculation (RH)	1	17.7	0.26
Cowpea x RH	1	0.63	0.08
Cowpea x Virus	4	4.11	0.92**
Virus x RH	4	8.14	1.12**
Cowpea x Virus x RH	4	22.41*	0.48
Error	24	10.4167	0.016111

** Significant at P≤0.01, ANOVA; * Significant at P≤0.01, ANOVA

Table 4. Analysis of variance table for weight of shoot and seeds greenhouse (experiment 2).

Variation	df	Shoot weight			Weight of roots		
		Sum of square	Mean sum of square	F	Sum of square	Mean sum of square	F
Cowpea (Block)	1	0.822	0.822	0.32	2.48	2.48	17.6**
V	4	63.84	15.96	6.213**	29.96	7.49	53.1**
Innoculation (RH)	1	0.54	0.543	0.21	0.01	0.01	0.07
Cowpea x RH	1	2.426	2.426	0.95	0.01	0.01	0.07
Cowpea X Virus	4	15.315	3.829	1.491	0.83	0.21	1.5
Virus X RH	4	41.581	10.395	4.05	0.73	0.18	1.3
Cowpea X Virus X RH	4	3.785	0.946	0.37	1.11	0.28	1.987
Error	24	61.65	2.56875		3.3816	0.1409	

**Significant at P≤0.01, ANOVA, * Significant at P≤0.01, ANOVA. V, Virus; RH, rhizobium; Cowp, cowpea and RH, rhizobium.

Table 5. Effects of varietal difference, rhizobial and viral inoculation on nodulation, vegetative and seed yield of cowpea.

Genotypes cowpea	Nodule number	Nodule weight (g)	Weight of sht (g)	Weight of seeds (g)
IF	8.29^{a}	0.36^{a}	2.61^{a}	2.44^{b}
OW	8.09^{a}	0.33^{b}	2.40^{a}	2.80^{a}
Rhizobial inoculant				
RH_0	7.72^{b}	0.35^{a}	2.42^{a}	2.61^{a}
RH	8.66^{a}	0.39^{a}	2.59^{a}	2.62^{a}
Virus inoculant				
V_0	9.88^{a}	0.39^{a}	2.77^{ab}	3.10^{a}
CYMV	9.36^{a}	0.37^{b}	2.31^{b}	2.83^{b}
CABMV	8.75^{a}	0.37^{ab}	2.68^{ab}	2.78^{b}
BICMV(IT16)	3.16^{b}	0.21^{c}	1.00^{c}	1.41^{c}
SBMV-Oyo	9.81^{a}	0.38^{ab}	3.77^{a}	2.95^{ab}
EMS	4.32	0.20	2.54	0.14

CYMV = Cowpea mosaic virus, CABMV = Cowpea aphid-borne mosaic virus, BICMV = Blackeye cowpea mosaic virus, SBMV = Southern bean mosaic virus, RH = Rhizobium, IF = Ife brown and OW = Owode

found in Table 6. Only dry weight of shoot of IF that was free of virus was significantly (p, 0.01) increased by RH-284. Number of nodules as well as weight of seeds of IF inoculated with CYMV was significantly enhanced by rhizobium RH-284. With CABMV on IF, dry weight of shoot was significantly increased by rhizobial inoculant. Even though Ife brown cowpea was infected with BICMV, the number of nodules was increased by RH-284. Number of nodules, weight of shoot and seeds of IF were significantly increased by RH-284 relative to un-inoculated

Table 6. Interactive effects of variety, rhizobial and viral inoculation on nodulation, vegetative and seed yield of cowpea.

Treatments	Nodule number	Nodule weight	Weight of shoot	Weight of seed
1.IFV$_0$RH	11.32^{abc}	0.4^{abc}	4.33^{ab}	2.49^{ef}
2. IFV$_0$RH0	8.98^{bcdef}	0.20^{cd}	3.52^{efg}	2.64^{cdef}
3. IFCYMVRH	7.62^{ef}	0.30^{bcd}	3.46^{efg}	2.56^{def}
4. IFCYMV	3.15^{g}	0.11^{d}	2.91^{g}	1.43^{g}
5. IFCABMVRH	7.60^{ef}	0.2^{cd}	3.99^{abcde}	3.00^{abcde}
6. IFCABMV	6.09^{gf}	0.2^{cd}	3.43^{efg}	3.43^{a}
7. IFBICMVRH	10.81^{abcd}	0.40^{abc}	4.19^{abcd}	2.86^{bcdef}
8. IFBICMV	8.04^{def}	0.28^{bcd}	3.58^{def}	3.14^{abc}
9. IFSBMVRH	3.16^{g}	0.12^{d}	2.12^{h}	1.43^{g}
10. IFSBMV	10.43^{abcde}	0.27^{bcd}	3.51^{efg}	3.13^{abc}
11. OWV0RH	10.27^{abcde}	0.33^{abc}	4.43^{a}	3.25^{ab}
12. OWV0	8.85^{cdef}	0.17^{cd}	3.41^{efg}	2.64^{cdef}
13. OWCYMVRH	9.62^{bcde}	0.28^{bcd}	4.24^{abc}	2.40^{f}
14. OWCYMV	4.14^{g}	0.10^{d}	1.56^{h}	1.41^{g}
15. OWCABMVRH	12.37^{a}	0.55^{a}	4.33^{ab}	2.60^{def}
16. OWCABMV	11.85^{ab}	0.19^{cd}	3.78^{bcdef}	3.25^{ab}
17. OWBICMVRH	8.80^{cdef}	0.17^{cd}	3.55^{efg}	3.19^{ab}
18. OWBICMV	9.69^{abcde}	0.16^{cd}	3.62^{cdef}	3.03^{abcd}
19. OWSBMVRH	3.17^{g}	0.10^{d}	1.67^{h}	1.41^{g}
20. OWSBMV	8.85^{cdef}	0.5^{ab}	3.32^{fg}	3.06^{abcd}

Standard error of mean. All values are averages of 4 replicates. Values followed by the same letters of the alphabet are not significant. CYMV = Cowpea mosaic virus, CABMV = Cowpea aphid-borne mosaic virus, BICMV = Blackeye cowpea mosaic virus, SBMV = Southern bean mosaic virus, RH = Rhizobium.

plant carrying SBMV.

On virus-free Owode genotype, dry weight of shoot and seed were significantly increased by RH-284 relative to uninoculated plant. However with CYMV, number of nodules, dry weight of shoot and seed were significantly increased relative to RH-284 free plant. Weight of shoot and seeds as well as nodule weight of CABMV-inoculated plant were significantly enhanced by rhizobium. Rhizobial inoculation had no positive effect on all the parameters of BICMV inoculated OW genotype but enhanced nodule number and weight as well as weight of shoot and seeds of SBMV inoculated OW.

DISCUSSION

Yield reduction or outright crop failure in cowpea resulting from viral attack are of common occurrence in sub-Saharan African. This has necessitated the need to carry out studies on biological strategy to reduce the incidence of cowpea viruses.

Results in the preliminary study have shown that the 2 viruses used had significant negative impact on the number of nodules in the 2 cowpea genotypes used. While no significant difference was observed in the 2 cowpea genotypes used with respect to their growth. Cowpea yellow mosaic virus (CYMV) and cowpea aphid borne mosaic virus (CABMV) significantly depressed nodule number. Infection of cowpea by these viruses led to impairment of vegetative growth with most of the leaves curling. This indicates that some physiological processes of growth might have been impaired. Nodulation, according to Denarie et al. (1996) and Spaink (2000) is a physiological process that involves the production of diffusible plant and bacterial metabolites such as flavonoids and lipo-chitooligosaccharides respectively, which trigger certain steps of the processes. It is assumed that interference of these processes had caused the reduction in nodule number. Effective nodulation also depends on the population of infective rhizobia in soils. Increasing the soil population through inoculation in this study had led to improved nodulation.

Significant increases in nodule number and root weight were observed in *Rhizobium* inoculated cowpeas. Legume inoculation is a process for the manipulation of rhizobial microflora for improving crop productivity and soil fertility (Keyser and Li, 1992). Although, rhizobial species are as widely distributed as the legumes themselves, there are many soils where suitable strains are absent, or where the population density is as low as to pose a threat to legume establishment and effective nodulation and N_2 fixation. The population of indigenous rhizobial in most tropical soils is very low and these indigenous strains are less efficient in fixing nitrogen

(Ahmad et al., 1981; Ahmad and Mclaughling, 1985). The selection and application of specific rhizobial inoculant as carried out in this study can be exploited in sustainable cowpea production. It was generally observed that nodule number and other growth parameters assessed were significantly reduced by BICMV (IT16). The infection caused by BICMV demonstrated this by resulting in significant growth and yield reductions in the cowpea cultivars used. This confirms previous results by Owolabi et al. (1988) which indicated that BICMV posed a more serious threat than any other virus to the production of cowpea cultivars. This was shown in more severe symptoms including death of plants at an early age in BICMV infections. Viruses such as CABMV and CYMV do cause severe symptoms including mosaic, green vein-banding, stunting and dramatic yield losses. The loss may range between 40-100% depending on the age of the plant at the time of infection (Chant, 1960; Raheja and Leleji, 1974). These results agree with those reported by Tu et al. (1970*)* and Hair and Miller (1982) working with clover and cowpeas, respectively. Patil and Sayyad (1994) reported a greater reduction in nodule number in virus infected cowpea than in *Rhizobium*-virus infected plants. They also reported that the reduction in the fresh weight of the nodules was greater than the reduction in fresh weight of plant due to CYMV.

On the interactive effect of RH-284 and viruses, inoculation of IF and OW with RH-284 significantly increased some parameters in spite of viral infection. This implies that virus infected plants produced more severe symptom of infection when they were not inoculated with rhizobium than *Rhizobium*-virus treated plants. The increase in nodule number and some other parameters of RH-284 inoculated plant relative to un-inoculated control indicated that the inoculant strain contributed significantly to nodulation. Eaglesham (1985) noted that it might be safer to rely on effective inoculant strain than breed plant for the ability to nodulate with indigenous strains of unknown potential. However, rhizobial inoculant can only be successful if it is more competitive than the native rhizobial in nodule formation and N_2 fixation.

Generally, in legume symbiosis, regulation of N_2-fixation is mediated by the host legume rather than by the bacterial symbiont (Giller and Wilson, 1993). In this study, infection by the viruses impaired some growth and yield parameters in spite of rhizobial inoculation. The inference drawn here is that it is only the severity of infection that can be reduced, infection cannot be stopped with rhizobium inoculation and not the infection of virus itself. The actual effect of increased nodulation and N_2-fixation was subsumed in the negative impact of virus attack, leading eventually to the complete suppression of the growth advantage conferred on the plants by the rhizobial inoculants.

There may be the need to experiment with various concentrations of the rhizobial inoculant, in order to derive maximum advantage from the positive impact of rhizobial inoculation.

REFERENCES

Ahmad MH, Mclaughling W (1985). Ecology and genetics of tropical Rhizobium species. Biotechnol. Advances. 3:155-170.

Ahmad MH, Eaglesham ARJ, Hassouna S (1981). Examining serological diversity of cowpea-rhizobia by the ELISA technique. Archives Microbiol. 130:281-287.

Chant SR (1960). The effect of infection with tobacco mosaic and cowpea yellow mosaic viruses on the growth rate and yield of cowpea in Nigeria. Empire J. Exp. Agric. 28: 114-120.

Denarie J, Debelle F, Prome JC (1996). Rhizobium lipo-chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. Annals Review Biochem. 65: 503-535.

Eaglesham ARJ (1985). Comparism of nodulation promiscuity of USA and Asian type soybean. Tropical Agric. (Trinidad) 62:105-109.

Giller KE, Wilson KJ (1993). The process of Nitrogen Fixation: In Nitrogen Fixation in Tropical Cropping Systems. *CAB Internataional*, Wallinford, UK.pp. 51-66.

Gilmer RM, Whitney WK, Williams RJ (1974). Epidemiology and control of cowpea mosaic in Western Nigeria. In: *Proceedings of First IITA Grain Legume Workshop*, IITA, Ibadan, Nigeria.

Hair SK, Miller JC (1982). Effect of virus infection on N-fixation in cowpea. Review Plant Pathol. 61(2):617

Keyser HH, Li F (1992). Potentials for increasing biological nitrogen fixation in soybean. Plant and Soil 141:191-135.

Nordam D (1973). Identification of Plant Viruses, *Methods and Experiments*. Centre for Agricultural Publishing and Documentation. Wageningen, Netherlands, 207pp.

Owolabi AT, Taiwo MA, Mabadeje SA (1988). Effects of single and mixed inoculations with blackeye cowpea mosaic and cowpea mosaic viruses on two Nigerian cowpea cultivars. *Nigerian Journal of Basic and Applied Sciences.* 2:25-33.

Patil PL, Sayyad AN (1994). Investigations into Nodulation and Leghaemoglobin Content of Cowpea as Influenced by Rhizobium-Virus Interaction. *In Virology in the Tropics* (Eds: Narayan Rishi, K. L. Ahuja and B. P. Singh. Malhora Publishing House, New Delhi 110 064, India. pp. 455-460.

Rachie KO (1985). Introduction. In: *Cowpea research, production and utilization* (SR Singh, K O Rachie, eds) John Wiley and Sons, Chichester: 25-30

Raheja AK, Leleji OI (1974). An aphid-borne virus disease of irrigated cowpea in northern Nigeria. *Plant Diseases Report.* 58:1080-1084.

Shoyinka SA (1974). Status of virus diseases of cowpea in Nigeria. In: *Proceedings of the First IITA Grain Legume Improvement Workshop*. IITA, Ibadan, Nigeria.

Singh SR (1980). Cowpea pest and diseases. IITA Ibadan, Nigeria.

Spaink HP (2000). Root nodulation and infection factors produced by rhizoidal bacteria. *Annals Review Microbiology*. 54:257-288.

Summerfield RJ, Dart PJ, Huxley PA, Eaglesham ARJ, Minchin FR, Day JM (1977). Nitrogen nutrition of cowpea (Vigna unguiculata), I. Effects of applied nitrogen and symbiotic nitrogen fixation on growth and seed yield. *Experimental Agriculture.* 13:129-142

Taiwo MA (2003) Viruses infecting legumes in Nigeria:case history In: Hughes J d'A, Odu,BO (eds). Plant virology in sub-Saharan Africa. *Proceedings of a conference organized by IITA Ibadan*, Nigeria. pp.365-380.

Thottappilly G, Rossel HW (1992) Viruses of cowpea in tropical Africa. *Tropical Pest Management* 38(4):337-348.

Tu JC, Ford RE, Grau CR (1970). Some factors affecting the nodulation and nodule efficiency in soybean infected by soybean mosaic virus c. *Phytopathology* 60:568-570.

Vincent JM (1970). *A manual for practical study of root nodule bacteria.* Blackwell scientific publication handbook 15 Oxford, UK.

Wells DG, Deba R (1961). Sources of resistance to the cowpea yellow mosaic virus. Plant Dis. Rep. 45:878-881.

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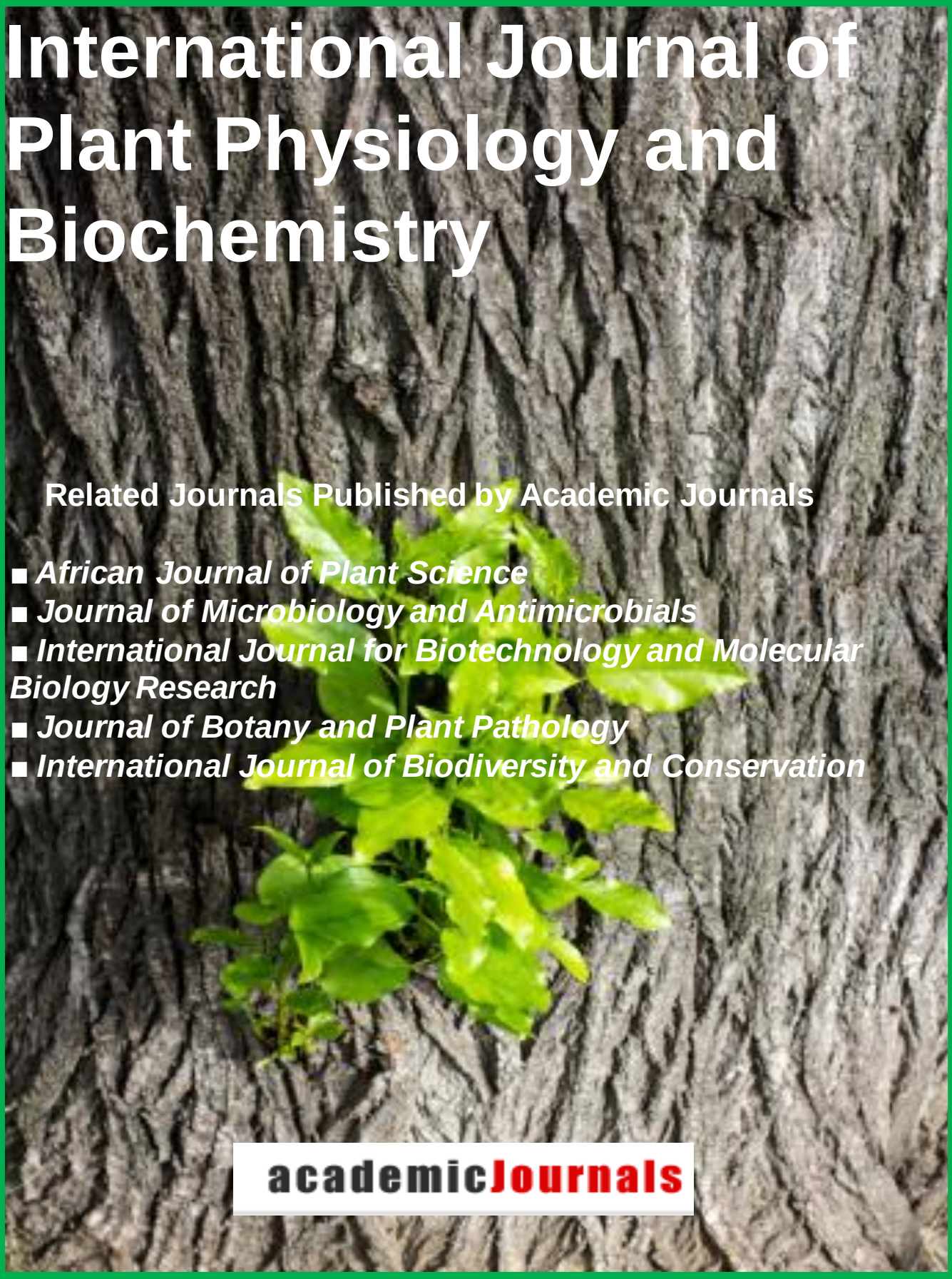

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