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Effect of carbon dioxide on seed transmission of *Lettuce mosaic virus*

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Abstract

Carbon dioxide as the cause of glasshouse effect and global warming is the current dilemma of human societies. It is well know that CO_2 is one of the principle requirements of plant growth and it promotes seed production. As to the seed transmission rate of virus under increased CO_2 level there was no work prior to this study. In this study, seeds harvested from lettuce plants that were grown under ambient (375 ppmv) and elevated (E) CO_2 (750 ppmv) and grown from *Lettuce mosaic virus* (LMV)-infected seeds or sap-inoculated were tested to find out if ECO_2 affects the seed transmission rate. Accordingly, two seed-lot samples (each 25 seeds) from each of the treatments were germinated on wet filter paper in Petri dishes and four-day old seedlings were subjected to double antibody sandwich enzyme-linked immunosorbet assay (DAS-ELISA) by the use of "home-made" conjugate with appropriate controls. The outcome from this study showed that the transmission rate in seeds from plants grown from infected seeds under ECO_2 was higher (8.5%) compared to that from plants grown under ambient CO_2 (6.8%). Likewise, the rate in seeds from sap-inoculated plant under ECO2 was higher (4.17%) than that from plants grown under ambient CO2 (4%). In addition, the transmission rates in plants grown from infected seeds were higher (8.5% or 6.8%) than that in seeds from the inoculated plants (4% or 4.17%).

Keyword: CO_2, ambient, transmission, LMV, seed, ELISA

اثر دی اکسید کربن روی انتقال بذری ویروس موزاییک کاهو

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چکیده

دی اکسید کربن (CO_2)، عامل اثر گلخانه‌ای و افزایش گرمای کرۀ زمین، به عنوان مشکل اصلی جوامع بشری امروز است. این گاز بعنوان یکی از نیازمندی‌های اساسی رشد گیاهی و افزایش دهندۀ تولید بذر شناخته شده است. دربارۀ اثر مقادیر بالای CO_2 (ECO_2) روی انتقال بذری ویروس های گیاهی، تاکنون هیچ مطالعه ای صورت نگرفته است. در این مطالعه، بذور حاصل از کاهوی روییده تحت مقدار عادی (۳۷۵ پی پی ام) و سطح بالای (۷۵۰ پی پی ام) CO_2 از بذور آلوده به ویروس یا مایه زنی شده با ویروس مورد بررسی گرفتند تا اثر ECO_2 بر انتقال بذری تعیین گردد. دو نمونۀ بذری (هر کدام ۲۵ بذر) از هر یک از گیاهان هر تیمار روی کاغذ فیلتری مرطوب در تشتک پتری جوانه زنی شدند و گیاهچه های چهار روزه با DAS-ELISA با کانژوگۀ تهیه شده آزموده شدند. نتایج نشان داد که انتقال در بذور حاصل از گیاهان روییده از بذور آلوده تحت ECO_2 بالاتر(۸/۵ ٪) از انتقال بذری در گیاهان رشد کرده تحت سطح معمولی CO_2 (۶/۸ ٪) بودند. همین‌طور، انتقال در بذور حاصل از گیاهان آلوده با شیره گیاهی، تحت ECO_2 بیشتر(۴٪/۱۷) ازبذور گیاهانی بود که تحت شرایط معمولی CO_2 رشد کرده بودند (۴٪). بعلاوه، میزان بذرزادی ویروس در گیاهان رشد کرده از بذور آلوده بیشتر (۸/۵ و ۶/۸ ٪) از مقدار آن در بذور حاصل از گیاهان آلوده شده با شیرۀ گیاهی بود (۴ و ۴/۱۷٪).

کلمات کلیدی: دی اکسید کربن، انتقال، ویروس بذر، الایزا، موزاییک، کاهو

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Introduction

Lettuce mosaic virus (LMV) is one of the first viruses reported in early 20th century (Jagger 1921 cited by Le Gall 2003) even before virus physicochemical property was known. LMV belongs to the genus *Potyvirus* that are transmitted by seeds, aphids and plant sap as well as vegetative propagation material. The virus particles are flexuous filamentous with 13 nm width and 746 nm length (Moghal & Francki 1981).

LMV is a serious pathogen of many commercial crops in lettuce- growing areas of the world. The pathogen causes losses in the field, but it is also a significant problem in glasshouses when seedlings are not grown under insect-proof conditions and are exposed to the infection. The severity of the disease is related to whether certified or uncertified seed is used and to the cultivation method. Serious losses occur when farmers are not obliged or unaware of the necessity to use certified seeds and particularly if relatively small plots are grown for successive years (Dianant & Lot 1992). Accordingly, because of the seed exchanges among different countries, LMV has widely spread all over the world. In Iran, LMV has been reported as the most dominant virus among three viruses detected in lettuce (Soleimani *et al*. 2004).

The virus has a relatively broad host range belonging to several families (German-Retana et al. 2008). Symptoms caused by LMV vary in different lettuce varieties. It causes dwarfing, defective heading, mottling, leaf distortion and yellowing in the lettuce variety Butterhead. ln crisphead varieties it produces blotching, dwarfing and leaf distortion particularly in the early stages. LMV-infected lettuce plant varieties of Cos are stunted and fail to make a compact heart (Dinant & Lot 1992).

Seed transmission of LMV was demonstrated in early years of virus discovery (Newhall 1923 cited by Le Gall 2003) when the virus entity, its nucleocapsid composition was yet to be explored. LMV is transmitted by both pollen and ovules but it is extremely low through pollen (Ryder 1964). LMV is seed-borne as it has been detected in hypocotyl, endosperm and radicle of the seed (Hunter & Bowyer 1991). The rate of virus transmission via seed depends upon the time of infection of the mother plant, the variety and the environmental conditions (Bos 1999). Accordingly, if plants are infected just before flowering, fewer virus-infected seeds are produced but no virus-infected seed is produced if the plants are infected after flowering. Although seed transmission is normally very low but from epidemiology point of view it is very critical. In Europe the acceptable threshold limit of lettuce seeds infected by LMV was 0.1%, until it came to light that even seed infection as low as 0.003% was enough to start an epidemic (Aveling 2014).

Climate change is a major concern in today's world, causing so-called glasshouse effect. Carbon dioxide (CO_2) as a major air pollutant is also one of the growth requirements for plants as it is involved in photosynthesis. It is well known that elevated CO_2 (ECO_2) in plant atmosphere increases the net photosynthesis and reduces photorespiration (Hew & Gibbs 1969), and inhibits the dark respiration through effects on mitochondrial metabolism (Shipway & Bramlage 1973).

There are conflicting reports as to the effect of ECO_2 on plant pathogens including viruses. Strengbom & Reich (2006) reported that incidence of leaf spot on mature leaves of *Solidago rigida* is reduced by half under ECO_2 whereas Kobayashi *et al*. (2006) found that in rice (*Oryza sativa* L.) both rice blast and sheath blight increase under ECO_2. In regard to plant viruses, because CO_2 affects plant cell physiology and plant virus becomes part of plant physiology during the infection (Bos 1999) there have been investigations into the effect of CO_2 on plant diseases (Luck *et al*. 2010; Lake & Wade 2009; Coakley *et al*. 1999). ECO2 generally favours the salicylic acid (SA) signalling pathway but represses the jasmonic acid (JA) pathway, which is associated with enhanced resistance to TMV (Zhang *et al*. 2015). Another study (Guo *et al*. 2015) reports that under ECO_2 tomato plants containing the R gene Mi-1.2 are more vulnerable to tomato yellow leaf curl virus (TYLCV) whereas

the variety Moneymaker defies the virus under ECO_2 suggesting that the effect of ECO_2 depends on plant-virus combination. Yet in another study (Trębicki *et al*. 2017) it has been reported that under ECO_2 the susceptibility of wheat plants against BYDV is increased.

The first investigation was in 1940s when increased CO_2 (1%) efficiently reduced susceptibility of *Phaseolus vulgaris* to tobacco necrosis virus (TNV) (Kalmus & Kassanis 1944). Later on, 1% ECO_2 efficiently inhibited tobacco mosaic virus (TMV)'s local lesions on tobacco plants and *N. glutinosa*, and that of turnip mosaic virus (TuMV) on tobacco plants (Hew & Gibbs 1969). So, along with positive effect of CO_2 on plant growth it appears to promote plant resistance to plant viruses (Matros *et al*. 2006). Moreover, exposure of oat plants to ECO_2 and simultaneous infection with *Barley yellow dwarf virus* (BYDV) promotes the plant growth (Malmström & Field 1997).

Therefore, the speculation was if ECO_2 can make plant resistant against virus by reducing or preventing local lesions it is anticipated that it might also affect seed transmission rate of virus. This is because the effect of CO2 on plant physiology is inevitable and, in turn, virus replication is governed by plant cell physiology which may in turn impact seed transmission rate of virus.

Materials and Methods

Lettuce plants were grown from seeds or inoculated with LMV-infected sap in chambers under conditions of ambient and elevated carbon dioxide, 375 ppmv and 750 ppmv, respectively. A seed sample with 13% infection was provided by Yates Pty Ltd. The infection in the mother seed had been verified by mechanical inoculation assay on *Chenopodium quinoa*. Healthy controls were grown from LMV-free seeds under ambient (AH) or ECO2 (HH). The plants were allowed to grow to maturity and the seeds from each plant (replicate) were collected in a small paper bag and kept at 4 °C until testing against LMV. The harvested seeds were germinated on pleated filter paper (Whatman No. l) in Petri plates (9 cm) at room temperature (~ 25 °C) and the plates were moved into a light room with 24h photoperiod to increase the growth rate of the seedlings. Every 4-day seedling (~15 mg) was extracted in a 1.5 ml sterilized micro-tube with a sterilized matching polypropylene pellet mixer (Knotes Biotech, New Jersey, USA) as a pestle in 700 µl extraction buffer (pH 7.4) containing 2.0% polyvinyl pyrrolidione (PVP), 0.2% bovine serum albumin (Albumin Fraktion V, Boehringer Mannheim) and 0.02% sodium azide (NaN3). First, each seedling was extracted in the absence of the buffer, then half of the extraction buffer, 350 µl, was added, followed by extraction and adding the remaining half of the buffer. Since LMV is an unstable virus (Ainsworth & Ogilvie 1939), the extracts were placed in ice for 15-30 min to proceed with all the extractions. The extracts were centrifuged and then placed back in the ice box before putting in the wells in ELISA microtiter plates.

In the first run of ELISA, a germinated certified LMV-free seed of cv. Salinas was used as virus-free control. In subsequent runs of ELISA, however, seedling extract from the first ELISA that was proved to be LMV-free was used as the control. Also, inoculated leaves of *Chenopodium quinoa*, expressing chlorotic local lesions were extracted and used as positive controls.

C. quinoa plants were inoculated as described elsewhere (Bos 1999). Briefly, freeze-dried LMV-infected leaves of *Pisum sativum* L. cv. Greenfeast (l g leaf in 10 ml buffer) were extracted in 0.01M phosphate buffer, pH 7.0 in a sterilized mortar with a sterilized pestle. Hands were washed with soap before rubbing the extract on carborundum- dusted leaves. As a healthy control, one plant was inoculated with healthy sap.

Antiserum against an Australian isolate of LMV, obtained from naturally infected lettuce at Hillston, NSW (Australia) was prepared in a rabbit. The gamma immunoglobulin (lgG) was separated from the antiserum through an affinity chromatography column (DEAE Affi-gel blue, BioRad). To prepare l ml of the conjugate, 100 µl of alkaline phosphatase (ALP) solution

(Bochringer Mannheim) was added to l ml of the anti-LMV lgG. Then, 8% glutaraldehyde solution was added to 0.06% (v/v) final concentration (8.5 µl glutaraldehyde to ll00 µl of the mixture), followed by incubation at room temperature for 2-3 h before dialyzing two times against 500 ml PBS (phosphate buffered saline) to remove glutaraldehyde. Finally, 5 mg/ml bovine serum albumin (Boehringer Mannheim) and 0.02% (w/v) NaN3 were added and stored at 4 °C.

A preliminary double antibody sandwich (DAS)-ELISA was performed according to Clark and Adams (1977) to determine the optimum dilutions of the coating IgG, sample, and the conjugate to be used in the ELISA. In the ELISA tests, 200-µl of IgG was placed in each well in the ELISA plate and incubated at 35 °C for 2 h to bind IgG to the well. Then, the plate was emptied into sink and tapped several times on paper towel to remove non-adsorbed IgG, followed by flooding with washing buffer (PBS-Tween) for three minutes, and then emptying and tapping on towel paper. This was repeated for three times. Samples and extraction buffer (control) were placed in the wells, followed by incubation at 4 °C overnight. After washing as above, 200-µl aliquots of the conjugate were placed into the wells, incubated at 35 °C for 2h and then washed as before. Then, four 5-mg p-nitrophenyl phosphate tablets (the substrate) were dissolved in 20 ml substrate buffer (1 mg/ml) and 250 µl were placed in the wells, plus the first column of the plate as baseline. The plate was kept in dark (draw) at room temperature and the light absorbance values at 405 nm wavelength were monitored every 15 min by a Titertek® Multiskan plate reader (Flow Laboratories, USA). Three replicate samples of each extract were placed in three successive wells in each column from top to bottom. Similarly, aliquots of extracts from healthy and infected controls were added into three successive wells in a column. The light absorbance at 405 nm of every plate was read by the plate reader. Twice mean absorbance values of virus-free extracts was considered as a threshold (Hill, 1984) so that if the mean value of a sample was more than the threshold it was considered as infected.

Results

The highest dilutions of the IgG, sap and conjugate were determined to be 0.1 µg/ µl, 1:100 and 1:1000, respectively, in the preliminary DAS-ELISA. When the test was carried out on the 4-day seedlings, clear cut results in terms of difference between positive and healthy seedlings were obtained. Accordingly, for majority of the tested seed samples the results were easily judged visually (Figure 1) even without using the ELISA plate reader although the plates were also scanned and read in the plate reader to obtain precise light absorption values of the treated wells at 405 nm wavelength.

The rate of infection in seeds from plants grown from infected seeds under ECO_2 (HNI) was higher than that in plants grown from the infected seeds under ambient CO_2 (ANI), 8.5% versus 6.8%, respectively. Likewise, the rate in seeds from sap-inoculated plants under ECO_2 (HI) was more than that in sap-inoculated plants under ambient CO_2 (AI), 4.17% against 4%, respectively. Also, the virus transmission rate in plants grown under ambient CO_2 from infected-seed (ANI) was higher (6.8%) than that from plants grown under similar condition but mechanically infected (AI) (4%). In addition, majority of the sap-inoculated plants grown either under ambient CO_2 or ECO2 (AI-92, AI-95, AI-96, AI-98, HI-63, H)-65, HI-66, HI-68) gave no infected seeds whereas in all the plants grown from the infected seeds there were infections except for HNI-59 (Table 1). Accordingly, the seed infection rate in ANI plants was higher than that in AI plants, 6.8% versus 4%, respectively. Likewise, in HNI plants (grown from infected seeds under ECO_2) there was 8.5% seed infection whereas in HI plants (inoculated plants under ECO_2) 4.17%.

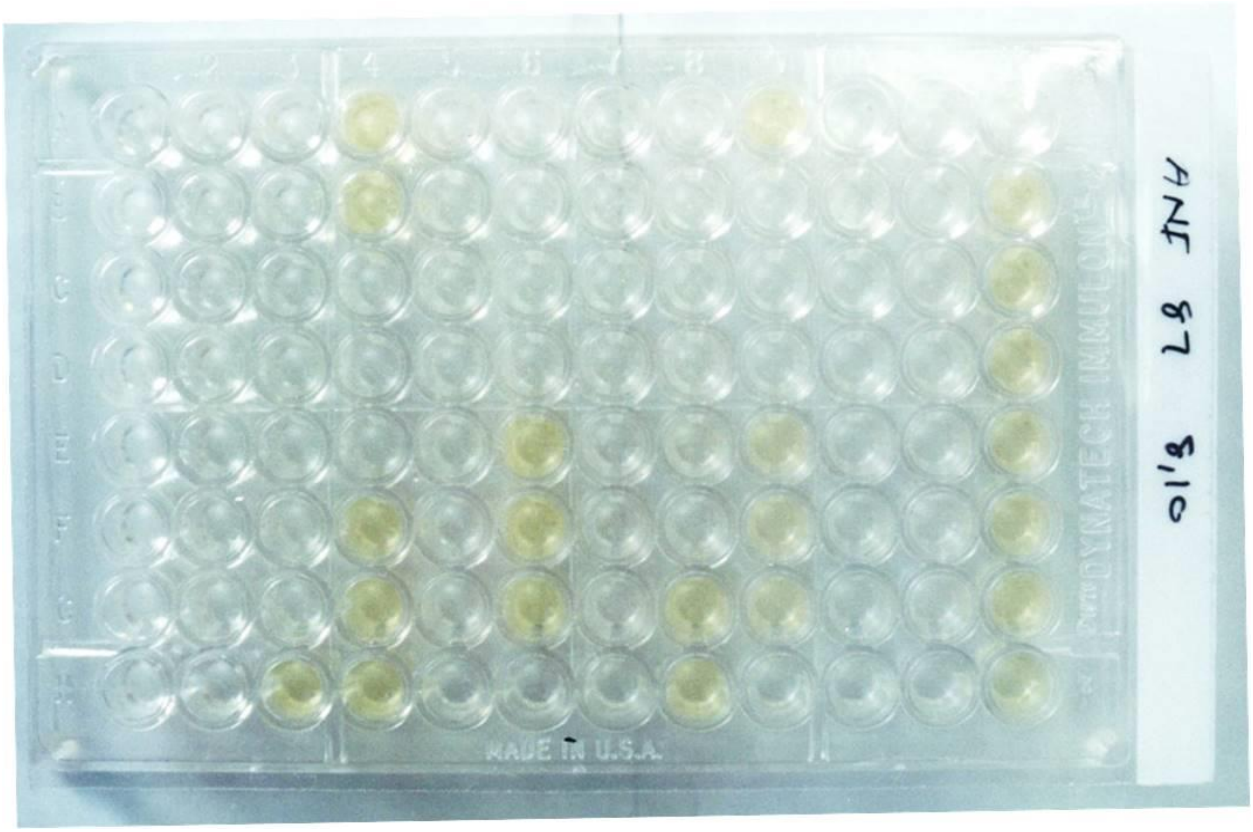

Figure 1. A representation of DAS-ELISA test with results from ANI-87 seedlings grown from *Lettuce mosaic virus*-infected seeds under ambient CO_2. Beginning from the second column three replicative aliquots of each sample were placed in three successive wells. For example, the sixth and eighth samples are infected. The last column is treated with the positive control, all showing yellow color. The results are clearly cut between healthy and infected seedlings, showing the reliability of the test.

Table 1. Number and infection rate with lettuce mosaic virus in seeds from plants grown under ambient or elevated CO_2 and infected from seed or sap inoculation.

Plant(s) [a]	Seeds tested	Number of infected seeds[b]		% infected seeds
		Sample lot 1	Sample lot 2	
ANI-85, -87, -88, -89 and ANI-91	250	6,5,0,1,0	1,1,1,1,1	6.8
HI-62, -64, -67, -70, -63, -65, -66, -68, -69, -71, -72 and HI-73	600	3,5,3,0,0,0,0,0,0,0,0,0	3,3,9,1,0,0,0,0,0,0,0,0	4.17
AI-97, -99, -101,-92, -95, -96, AI-98	350	5,5,0,0,0,0,0	2,0,2,0,0,0,0	4
HNI-57, -58, -59, HNI-61	200	2,3,0,3	5,3,0,1	8.5
HH-50, -51, -52, -54 and HH-55	250	0	0	0
AH-80, -81, -82, -83 and AH-84	250	0	0	0

[a] AI: ambient CO2- seeds from mechanically inoculated plant, HI: elevated CO_2- seed from mechanically inoculated plant, HH: elevated CO_2-seed from healthy plant, AH: ambient CO_2- seed from healthy plant, HNI: elevated CO_2-seeds from plant grown from infected seed, ANI: ambient CO_2- seeds from plant grown from infected seed

[b] Number of infected seeds are written respective to the treatment, e,g, ANI-85 and -87 had respectively 6 and 5 infected seeds in the lot1 and 1 and 1 in the lot 2.

Discussion

In this study hundreds of seeds from plants grown under ambient or elevated CO_2 from LMV-infected seeds or mechanically-inoculated plants were analyzed by the use of a very reliable DAS-ELISA. The preliminary DAS-ELISA test not only revealed optimal dilutions of the reagents but also demonstrated the efficiency of the "home-made" conjugate in detecting LMV. Although in the preliminary test, yellow color appeared in all the

wells within 5 h of adding the substrate, there were still cut results among the rows with different dilutions. Monitoring of the color development in an ELISA plate is normally done within 1-2 h from adding the substrate because eventually all the wells would turn into yellow due to aging of the substrate. Therefore, the reliability of the color development is normally in early hours of the adding the substrate which depends on virus quantity and consequently absorbance values. As long as the healthy control and the only- substrate-treated wells have not developed the color, the monitoring is still valid. In this preliminary test, the judgement by relying on the color development was still valid even after 5 h of adding the substrate.

Differences were revealed between the transmission rates of the seeds from the treated plants under high level (ECO_2) and ambient CO_2 and those from healthy controls (HH and AH). The transmission rates in plants exposed to ECO_2 were higher than that in seeds from plants grown under ambient CO_2. Accordingly, the rates in HNI and HI plants (ECO_2) were 8.5% and 4.17% whereas in ANI and AI plants (CO_2) they were 6.8% and 4%, respectively. This suggested that ECO_2 increases the virus infection rate in the seeds. This likely enhancing impact of ECO_2 in the virus seed transmission rate may be attributed to its increasing effect on seed production (Holley *et al*. 2022; Lamichaney et al. 2021; Way et al. 2010; Edwards *et al.* 2001). According to Holley *et al*. (2022), increasing carbon dioxide between 400 and 800 ppm results in lettuce fresh and dried weight. Although there is no report, so far, as to the boosting effect of ECO_2 on seed production in lettuce, there are such reports in other crops (e. g., Lamichaney *et al*. 2021). So, it is likely that ECO_2 can also promote seed production in lettuce. Then, there would be a scenario of the more seeds the more infected seeds. There are conflicting reports as to the effect of ECO_2 on plant-virus interactions, however it increases the susceptibility of wheat plants to barley yellow dwarf virus (BYDV) or cereal yellow dwarf virus (CYDV) (Trębicki *et al*. 2017). This seems to be in line with the conclusion drawn here because higher rate of the seed transmission can also be considered as being more susceptibility to the virus. Additionally, in the report by Aguilar *et al*. (2015), ECO_2 has intensified the synergism between potato virus X (PVX) and potyvirus, the same genus that LMV belongs to. This also seems to support the conclusion that ECO_2 may increase seed transmission of LMV.

Moreover, the infection rates in the seeds from plants grown from infected seeds were more than that from mechanically-inoculated plants. The infection rates in seeds from HNI and ANI plants (grown from infected seeds) were 8.5% and 6.8% whereas in HI and AI plants (sap-inoculated) 4.17 and 4%, respectively. Such differences could be attributed to the effect of duration of infection in mother plant on the seed transmission rate. This had been reported for the seed transmission rate of bean common mosaic virus (Bennett 1969). He noticed that the bean plants grown from the infected seeds had higher percentages of infection than the plants which were inoculated during vegetative growth stage. Fajardo also observed that there were no infected seeds from pods set prior to infection of the mother plant. It has been concluded that the seed transmission rate of bean mosaic virus was dependent on the ability of the virus to reach the ovule before or just after fertilization (Bennett 1969). The impact of time length of infection on the seed transmission rate has also been reported by Couch (1955) who demonstrated that the plants inoculated at 4-week-old had a higher seed transmission rate (7.82%) than that inoculated at 9- or 15-week-old, 4.91% and 4.63%, respectively. Also, plants inoculated after flowering did not have any seed transmission of the virus. These data suggest that after infection, virus needs time to multiply, pass through tissues, then to infect anthers and ovule, and finally reach the seed. Therefore, when the infection occurs earlier, the virus multiplies more, and it seems there is more chance of arrival and localization of LMV particles in the seed.

The rate of virus seed transmission is usually small under 0.1% (Bos 1999), however even a tiny rate will have much bigger consequences in plant virus epidemiology if the cumulative increase in seed transmission in successive years is taken into account, particularly if farmers use seeds from their own crop of previous year.

Two results are put out from this study. First, ECO_2 seems to increase LMV transmission rate in lettuce seeds. Second, the infection rates in seeds from plants grown from infected seeds are higher than that in plants inoculated with the infected sap.

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References

Aguilar E, Allende L, del Toro FJ, Chung B-N, Canto T, Tenllado F, 2015. Effects of elevated CO_2 and temperature on pathogenicity determinants and virulence of Potato virus X/potyvirus-associated synergism. *Molecular Plant -Microbe Interactions* 28: 1364–1373.

Ainsworth EA, Long SP, 2005. What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. *New Phytology* 165: 351–372.

Ainsworth GC, Ogilvie L, 1939. Lettuce mosaic. *Annals of Applied Biology* 26: 279–297.

Aveling TAS. Global standards in seed health testing. In: Gullino ML, Munkvold G, editors. Global Perspectives on the Health of Seeds and Plant Propagation Material. Dordrecht, Springer, 2014. p. 17–28.

Bennet CW, 1969. Seed transmission of plant viruses. *Advances in Virus Research* 14: 221–261.

Bos, L. 1999. Plant viruses, unique and intriguing pathogens - a textbook of plant virology. Backhuys Publishers, Leiden, The Netherlands. 358 pp.

Chen D, Mei Y, Liu Q, Wu Y, Yang Z, 2021. Carbon dioxide enrichment promoted the growth, yield, and light-use efficiency of lettuce in a plant factory with artificial lighting. *Agronomy Journal* 113: 5196–5206. DOI: 10.1002/agj2.20838.

Clark MF, Adams AN, l977. Characteristics of the microplate method of ensyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475–433.

Coach HBA, 1955. Studies on seed transmission of lettuce mosaic virus. *Phtopathology* 45: 63–70.

Coakley SM, Scherm H, Chakraborty S, 1999. Climate change and plant disease management. *Annual Review of Phytopathology* 37: 399–426.

Dinant S, Lot H, 1992. Lettuce mosaic virus. *Plant Pathology* 41: 528–542. https://doi.org/10.1111/j.1365-3059.1992.tb02451.x

Edwards GR, Clark H, Newton PCD, (2001) The effects of elevated CO2 on seed production and seedling recruitment in a sheepgrazed pasture. *Oecologia* 127: 383–394.

Fajardo TG, 1928. Progress on experimental work with the transmission of bean mosaic. *Phtopathology* 18: 155.

German-Retana S, Walter J, Le Gall O, 2008. Lettuce mosaic virus: from pathogen diversity to host interactors. *Molecular Plant Pathology* 9 (2): 127–136. doi: 10.1111/j.1364-3703.2007.00451.x.

Guo YP, Guo DP, Peng Y, Chen JS, 2005. Photosynthetic responses of radish (Raphanus sativus var. longipinnatus) plants to infection by turnip mosaic virus. *Photosynthetica* 43: 457–462.

Hew CS, Gibbs M, 1969. A study of chloroplasts of corn, sorghum, and sugar cane. *Plant Physiology* 44: 5–47.

Guo HJ, Sun YC, Li YF, Liu XH, Zhang WH, Ge F, 2014. Elevated CO2 decreases the response of the ethylene signaling pathway in *Medicago truncatula* and increases the abundance of the pea aphid. *New Phytologist* 201: 279–291.

Hill SA 1984, Methods in Plant Virology. Blackwell Scientific Publications, Oxford.

Holley J, Mattson N, Ashenafi E, Nyman M, 2022. The Impact of CO2 Enrichment on Biomass, Carotenoids, Xanthophyll, and Mineral Content of Lettuce (Lactuca sativa L.). *Horticulturae* 8: 820–831. doi.org/10.3390/horticulturae8090820

Hunter DG, Bowyer JW, 1991. Location of lettuce mosaic virus in mature lettuce seed tissues by immunogold cytochemistry. *Australasian Plant Pathology* 20:3–5. https://doi.org/10.1071/APP9910003

Kalmus, H. Kassanis B, 1944. Reduction by carbon dioxide of susceptibility of beans to tobacco necrosis viruses. Nature (London) 154: 641–642.

Jagger IC, 1921. A transmissible mosaic disease of lettuce. *Journal of Agricultural Research* 20: 737–741.

Kobayashi T, Ishiguro K, Nakajima T, Kim HY, Okada M, *et al*., 2006. Effects of elevated atmospheric CO2 concentration on the infection of rice-blast and sheath blight. *Phytopathology* 96: 425–431.

Lake JA, Wade RN, 2009. Plant-pathogen interactions and elevated CO2: morphological changes in favour of pathogens. *Journal of Experimental Botany* 60(11): 3123–3131. doi:10.1093/jxb/erp147

Lamichaney A, Tewari K, Basu PS, Katiyar PK, Singh NP, 2021. Effect of elevated carbon-dioxide on plant growth, physiology, yield and seed quality of chickpea (*Cicer arietinum* L.) in Indo-Gangetic plains. *Physiology and Molecular Biology of Plants* 27(2): 251–263. doi: 10.1007/s12298-021-00928-0.

Le Gall O, 2003. Lettuce mosaic virus. Description of Plant Viruses, no. 399, Association of Applied Biologists, UK.

Luck J, Aurambout J, Finlay K, Chakraborty S, Kriticos D, *et al*. 2010. An integrative approach to understanding the pest and disease threats to agricultural biosecurity under future climates. *9th European IFSA Symposium*, 4- 7 July, Vienna, Austria. PP. 1379-1388.

Malmström CM, Field CB, 1997. Virus induced differences in the response of oat plants to elevated carbon dioxide. *Plant Cell Enviroment* 20:178–88.

Matros A, Amme S, Kettig B, Buck- Sorlin, GH, Sonnewald U et al, 2006. Growth at elevated CO_2 concentrations leads to modified profiles of secondary metabolites in tobacco cv. SamsunNN and to increased resistance against infection with potato virus Y. *Plant, Cell & Environment* 29: 126–137.

Moghal SM, Francki RIB, I931. Towards a system for thc identification and classification of potyviruscs. II. virus particle length, symptomatology and cytopathology of six distinct viruses. *Virology* 112: 210–216.

Nelson R, 1932. Michigan Agricultural Station Technical Bulletin 118: 3–71.

Newhall AG, 1923. Seed transmission of lettuce mosaic. *Phytopathology* 13:104–106.

Purhoit AN, Tregguna EB, Ragetli HWJ 1975. CO2 effects on local-lesion production by tobacco mosaic virus and turnip mosaic virus. *Virology* 65: 558–564.

Rai P, Chaturvedi AK, Shah D, Pal M, 2016. Impact of elevated CO2 on high temperature induced effects in grain yield of chickpea (*Cicer arietinum*). *Indian Journal of Agricultural Science* 86(3):414–417.

Ryder EJ, 1973. Seed transmission of lettuce mosaic virus in mosaic resistant lettuce. Journal of *The American Society for Horticultural Science* 98: 610–614.

Shipway MR, Bramilage WJ, 1973. Effects of carbon dioxide on activity of apple mitochondria. *Plant Physiology* 51: 1095–1098.

Soleimani P, Mossahebi GH, Koohi-Habibi M, Zad J, Hosseini-Farhangi S, 2004. Occurrence and distribution of lettuce mosaic disease in Tehran province from Iran. *Communications in Agricultural & Applied Biological Sciences* 69(4): 513-7. PMID: 15756832.

Strengbom J, Reich PB, 2006. Elevated [CO_2] and increased N supply reduce leaf disease and related photosynthetic impacts on *Solidago rigida*. *Oecologia* 149: 519–525.

Trębicki P, Nancarrow N, Bosque-Pérez NA, Rodoni B, Aftab M, Freeman A, Yen A, Fitzgerald GJ, 2017. Virus incidence in wheat increases under elevated CO2: A 4-year study of yellow dwarf viruses from a free air carbon dioxide facility. *Virus Research* 241: 137–144.

https://doi.org/10.1016/j.virusres.2017.06.027.

Zhang S, Li X, Sun Z, Shao S, Hu L, et al., 2015. Antagonism between phytohormone signaling underlies the variation in disease susceptibility of tomato plants under elevated CO_2. *Journal of Experimental Botany* 66: 1951–1963. doi: 10.1093/jxb/eru538.

Way DA, Ladeau SL, Mccarthy HR, Clark JS, Oren R, Finzi AC, Jackson RB, 2010. Greater seed production in elevated CO2 is not accompanied by reduced seed quality in *Pinus taeda* L. *Global Change Biology* 16:1046-1056.