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Genetic Evaluation Study of Fava Bean Vicia Faba L. Under the Influence of the Transfer and Diagnosis of the Bean Yellow Mosaic Virus in Several Areas of Kirkuk Governorate

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Abstract

The results of the study and survey in the agricultural fields in Kirkuk Governorate, planted with the bean crop during the agricultural season (2022-2023) and (2023-2024) showed that the bean yellow mosaic virus was found in the fields of (Hawija - Daquq - Dibs) (Tu & Ford, 1968). The results showed that the highest infection rate with BYMV virus reached 48% in the pre-flowering stage in Hawija in the season (2022), while the lowest infection rate was 27% in Daquq. As for the 2023 season, the highest infection rate was in the Hawija area, reaching 52%, while the lowest was in the Daquq area, reaching 33%. The results of the serological diagnosis of the isolate obtained from the fava bean plants using the Tissue Blot Immuno Assay (TBIA) test showed that the main cause of mosaic symptoms on the fava bean plant included in the study is the BYMV virus, whereby the test showed the characteristic of the blue-purple distinct colour of the reaction on the nitrocellulose membrane, the soaked seeds in water after cutting and printing on the nitrocellulose membranes proved the presence of the BYMV virus, which is transmitted by fava bean seeds at a rate of 4%. As for the effect of treatments on the chlorophyll content of fava bean leaves, the statistical analysis showed significant differences between the treatments, as the plant barrier had a clear effect in increasing the amount of chlorophyll or maintaining the amount of chlorophyll a, b and total in fresh fava bean leaves, as its average amount reached (34.11), (30.02) and (25.93) mg/g, respectively. As for the number of nutrients in the leaves of fava bean plants,

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it was in a direction of decrease for the element's calcium, potassium and sodium and increase for the element nitrogen for infected plants, as the element potassium reached (1.81) mg, sodium (1.843) mg, calcium (1.732) mg, and nitrogen (1.567) mg.

Keywords:

Viruses, BYMV, seed transmission, broad bean, fava bean.

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Introduction

Broad bean (vicia faba L.) is a winter legume crop. Studies indicate that the Mediterranean basin is the original home of the broad bean. Its seeds contain a high percentage of protein and amounts of sugars, starches and some vitamins. It is used as animal feed (Al-Barzanji & Mahmoud, 2005) and its role is to improve soil properties by fixing atmospheric nitrogen through root nodule bacteria that coexist with it (Ilić et al.,2019) (Hasan & Abdullah, 2020).

China is one of the largest countries in the production and consumption of fava beans, followed by Ethiopia. China's fava bean production reaches (2.7 million tons/year), followed by Ethiopia 9%, then Egypt, which produces the equivalent of 262 thousand tons per year (Anonymous, 2003). In Iraq, the area planted with fava beans reached (17727) dunums, and the total production reached (25711) tons of seeds (Central Organization for Statistics and Information Technology and Agricultural Reports, 2022)

The fava bean crop is infected with about 50 viruses, including the Bean yellow mosaic virus (Kaiser, 1973). The virus infects a wide family range of crops, including legumes (fava beans, lentils, beans, and cowpeas), cucumbers, peppers, and cucurbits... etc (Mali et al, 2003). This depends on the variety, strain, and time of infection (Phatak, 1974).

Broad bean mosaic disease is one of the most widespread viral diseases in broad bean fields in Iraq and neighbouring Arab countries. This disease may be caused by one or more viruses (Kafi and Ayyash, 2022; Al-Jubouri et al., 2024) Broad bean, like other crops, is also affected by many diseases, most notably viral diseases. Many of them have been recorded in different regions of the world (Tolba, 1980). Due to their transmission in seeds from one season to another, these viruses cause economic losses, in addition to the availability of vector insects, the most important of which are aphids that transmit these viruses and spread them in the fields (Vij & Prashant, 2024; Phatak, 1974).

Materials and Research Methods

The study was implemented during the winter season (2022-2023) and (2023-2024) in farmers' fields spread in Kirkuk Governorate, namely (Hawija - Daquq - Dibs). Samples of up to (50) were collected using the perpendicular diameter method. Samples were collected from plants in the pre-flowering stage and printed on nitrocellulose membrane. Drying and preserving them until they are discovered to produce fava bean plants and inoculating them with samples obtained from the fields (Hasan & Abdullah, 2023). The fava bean seeds were planted after soaking them in water for 30 minutes in clay anvils containing soil previously sterilized with 1% formalin by adding 10 ml/kg soil and covered with a nylon cover for a week. After that, the cover was removed and they were stirred and left for a week before use. The weight of the soil in each anvil was 5 kg. After the seedlings germinated, they were placed under observation for insect infestation, especially aphids

and whiteflies, and they were controlled using the 50% noise pesticide by fumigation and also using the 50% prior pesticide at a rate of 5 g/liter by spraying on the plants. The samples were taken from the plants that were printed on nitrocellulose films and placed in nylon bags and the information was recorded on them (collection area, collection date, nature of the symptoms appearing on the plants, growth stage) and kept in a refrigerator until diagnostic tests were performed on them.

Serological Diagnosis of the Virus by the TBIA Method

The method described by (Makkouk & Kumari, 1996) was used in this test.

Serological Diagnosis of the Virus by the Diffuse Spot Method

The method described by (Tolba, 1980) was used.

Mechanical Inoculation to Study the Internal Symptoms of the BYMV Virus

Samples were taken from young leaves of fava beans that showed clear mosaic symptoms and were cut into small pieces using sterile scissors and crushed in a sterile ceramic mortar in the presence of a quantity of phosphate buffer solution (Na2 HPO4 .2H2O) + KH2 PO4) in a ratio of 2:1 weight: volume and at a concentration of 1% ml and a neutral pH of 0.2% of Sodium Diethyl at a rate of 1 ml/g plant and the extract was filtered through two layers of sterile muslin cloth and the filtrate was used as a source of inoculum (Humada et al., 2024). The process of inoculating fava bean plants at the age of 3-4 leaves with the extract was carried out after washing the hands well with soap and water and drying them with paper previously sterilized in the oven. The mechanical inoculation process was carried out using the finger method by dipping the index finger in the inoculum and wiping the upper surfaces of the plants covered with the abrasive material carborundum 400 mesh, then the inoculated leaves were washed directly with water After inoculation and left until symptoms appear (Noordam, 1973) and the result is consistent with (Abdullah & Hasan, 2020).

To study the internal symptoms resulting from infection with the virus in the cells of the leaflets of fava beans, a method was followed to detect macrophages by making parallel skin cuts to the midrib on the lower surface of an infected fava bean leaf at the beginning of the appearance of symptoms on the leaves of the inoculated plants using a sharp blade. (Hasan et al., 2024) After that, the skin was removed using pointed forceps prepared for this purpose and placed in a watch bottle containing a solution of Rose Bengal dye at a concentration of 0.5% for 5 minutes. After that, it was washed with distilled water and examined under a light microscope using a maximum magnification of 40×. For comparison, other skin cuts from healthy fava bean leaf epidermis cells prepared in the same way were examined (Noordam, 1973).

Testing the Transmission of the Yellow Bean Mosaic Virus in Fava Bean Seeds

After the emergence of seedlings, at an age of 3-4 leaves, the fava bean plants were inoculated with BYMV. After the infection appeared on the fava bean plants, the seeds of the infected fava bean plants were collected, dried and preserved, then soaked in water for two hours (Nayak & Raghatate, 2024). Then the seeds were cut in the Ruwisha region with blades (blade/seed) and printed on nitrocellulose membranes. These membranes were preserved until they were examined. They were diagnosed by the TBIA plant tissue fingerprint test. The result is consistent with (Hasan *et al.,* 2022).

The Effect of Mineral Oil Treatments, the Insecticide, and the Plant Barrier On

A field was selected in Al-Hawija district. The field was divided into 6 plots, each plot was divided into 10 subplots, then each plot was planted with 15 plants, and 5 plots were selected for each treatment. These treatments were as follows: number (1) was given to plants treated with distilled water only, number (2) to plants treated with mineral oil (Sunco), number (3) to plants treated with mineral oil + insecticide (alpha-methrin-cypermethrin), and number (4) to plants treated with insecticide. These treatments were distributed randomly. The plants were inoculated at the age of (6-8) leaves and left until fully mature until the end of the season when they were completely uprooted and placed in bags. As for the plant barrier, two plots planted with broad beans were chosen and surrounded by two alternating lines of yellow corn plants in the shape of the letter L in the direction of the wind, i.e. in the northwest direction.

Chlorophyll Content in the Leaves of Faba Bean Plants Infected with BYMV Virus

The amount of chlorophyll a, b and total was estimated by taking 1 g of leaves for each treatment according to three replicates then grinned in a ceramic mortar with 25 ml of cooled acetone (4°C at a concentration of 80%), then distributing it into tubes and then placing them in a centrifuge device at a centrifugal force of 2500 rpm for 30 minutes. The product was taken and the volume was completed to 25 ml by cooled acetone. The absorbance was measured by a Spectrophotometer at a wavelength of (663 - 645) nm, and then the amount of chlorophyll a, b and total was calculated according to the equation developed by (Ramadan, 1989).

Mineral Elements in the Leaves of Faba Bean Plants Infected with BYMV Virus

0.5 g of dried faba bean leaves were weighed for each treatment. The samples were digested by adding 10 ml of perchloric acid in a 50 ml glass beaker and left until the next day at a rate of Three replicates for each sample (Gresser & Parsan, 1979) The concentrations of potassium, sodium and calcium were measured using atomic absorption spectrophotometer type RYE Unicamspatatomic Absorption in the research laboratory after making the necessary dilutions on the standard curves for each element, then reading the absorbance of the elements through the wavelength of each element to obtain the concentrations of the elements in the plant. Total nitrogen was estimated after drying the plant samples at a temperature of 70 C for 48 hours in the oven according to what was stated in (A.O.A.C, 1980) using the Microkjeldahl method.

Results and Discussion

Field Survey

The results of the fields - survey planted with fava beans in Kirkuk Governorate during the agricultural season of (2022-2023) and (2023-2024) showed the presence of the virus of the type of Bean yellow mosaic virus. Table (1) shows during the season of (2022-2023) that the highest infection rate with the BYMV virus amounts to 48% in the pre-flowering stage in Hawija, while the lowest infection was 27% in Daquq. As for the season (2023-2024), the highest infection rate was in the Hawija area at 52%, while the lowest was in the Daquq area, reaching 33%. Thus, it becomes clear to us that the results indicate the widespread of this virus in fava bean fields, which is an expected result because the virus is transmitted by seeds along with by types of aphids that transmit it with high efficiency and in a non-persistent way, which has established the cultivation of this crop in these areas (Hawija - Dibs - Daquq) since a long time ago, which caused the establishment of pathogens that infect it, including this virus. Also, the increase in agricultural density for other crops is a storehouse for viruses and their insect vectors, especially aphids, as well as the wide host range of this virus, which leads to an increase in infection rates.

Table 1. The percentage of plants infected with the yellow bean mosaic virus

Season Percentage of BYMV Infection for the 2023-2024 Season	Percentage of BYMV Infection for the 2022-2023	Fields Location
52	48	Alhwayjah
41	38	Al-Dibs
33	27	Daquq

Serological Diagnosis of BYMV Virus

TBIA Test

The results of serological diagnosis of the isolate obtained from infected faba bean plants using the Tissue Blot Immuno Assay (TBIA) test showed that the main cause of mosaic symptoms on faba bean plants included in the study is the BYMV virus, as the test showed the characteristic blue-purple colour of the reaction on nitrocellulose membrane as shown in Figure (1 and 2) and that this test is widely used in diagnosing viruses in general, including viruses that infect plants such as BYMV virus. It is a rapid and sensitive test and is used with germinated seeds as well as from tender plant tissues. This method was also used in diagnosing BYMV virus by (Al-Barzanji & Mahmoud, 2005).

Virus Isolation

The results of mechanical inoculation on faba bean plants inoculated with the selected isolate in the way mentioned above led to diagnosing BYMV virus using the plant tissue fingerprint test TBIA as shown in Figure (2).

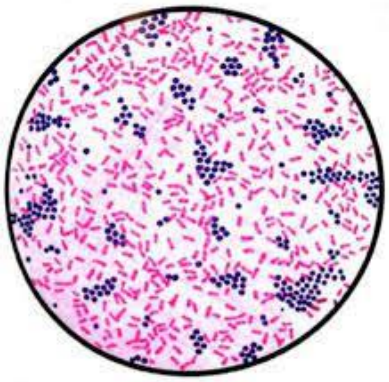

Figure 1. Results of plant tissue fingerprint test on nitrocellulose membrane for bean yellow mosaic virus (healthy plant fingerprint, green colour and infected plant fingerprint, purple colour)

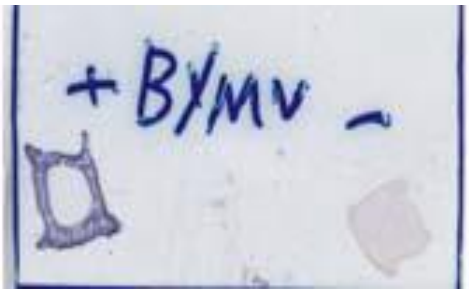

Figure 2. Plant tissue fingerprint test on nitrocellulose membrane that is infected with BYMV of a faba bean stem. Cross section of stem

Dot-Blot ELISA Test

The test showed the presence of the BYMV virus in the infected samples (Figure 3). The reaction was read by the naked eye and the blue-purple colour indicated the infected samples (positive) and the green colour or no colour appeared on the healthy samples (negative).

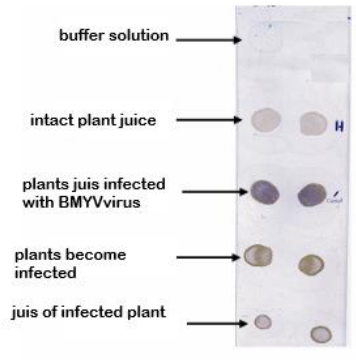

Figure 3. Dot-Blot ELISA test for detection of BYMV virus in samples

Mechanical Transfer of the Virus

The mechanical transfer of the virus from infected to healthy faba bean plants was successful. Systemic symptoms appeared on the inoculated plants in the form of vein transparency on the new leaves after (7-10) days of inoculation, afterwards appearance of pale green areas on the leaf blade accompanied by the appearance of irregular dark green areas distributed on the leaf blade, sometimes arranged around the main veins giving the appearance of vein banding. Some newly formed leaves in infected plants are observed to have deformities and small sizes. Some researchers have resorted to studying the family range to diagnose the virus, including, those who said that the symptoms are in the form of pale-yellow areas on the veins after (6-9) days of infection, followed by the appearance of pale yellow or green areas on the blade as the infection occurred early with severe deformation of the apical leaves and dwarfing of the infected plants. Found that the symptoms of the virus on fava beans appeared as yellow mottling, vein necrosis, leaf curling and plant stunting, while, showed that the virus affects fava beans and causes short internodes and the plant becomes shrubby, as the symptoms of the disease begin with the leaflets bending downwards and the appearance of yellow spots on newly growing leaflets also their surface becomes irregular (Leema & Balakrishnan, 2024)

Examination by light microscope of many fava bean leaf skin exfoliations showed mosaic symptoms of the presence of irregularly shaped granular bodies in the cytoplasm of epidermal cells and guard cells attached to or near the nucleus and darker in colour than the nucleus. Such bodies were not observed in the cells of exfoliations taken from healthy fava bean leaves, as the presence of macrophages in the cytoplasm of infected cells supports the diagnostic results, as these bodies appear with the group of potato viruses (Alatawi et al., 2024).

Seed transmission: Seeds soaked in water after cutting and printing on nitrocellulose membranes proved the presence of the BYMV virus, which is transmitted by fava bean seeds at a rate of 4%, as shown in Figure (4). This result is consistent with what was found by (Dallwitz et al., 2004).

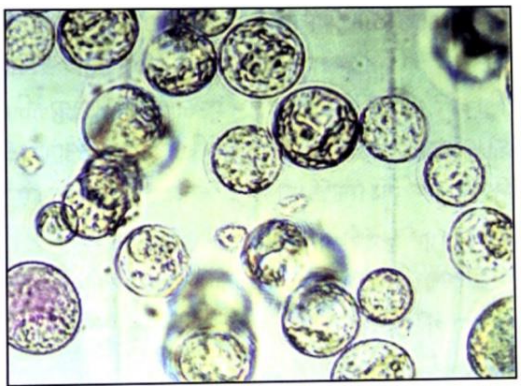

Figure 4. Transmission of BYMV by fava bean seeds (arrows indicate positive samples)

Effect of Mineral Oil, Insecticide and Plant Barrier Treatments On

1- Chlorophyll content in fava bean leaves that are infected with BYMV

Table (2) shows the effect of treatments on the chlorophyll content of fava bean leaves. Statistical analysis shows significant differences among treatments. The plant barrier had a clear effect in increasing the amount of chlorophyll or maintaining the amount of chlorophyll a, b and total in fresh fava bean leaves. Its average amount reached (34.11), (30.02) and (25.93) mg/g, respectively. It gave the best amount, followed by the mineral oil treatment with the insecticide whereby The amount of chlorophyll a, b and total was (24.84), (23.81) and (21.9) mg/g, respectively, compared to infected plants whereby The amount of chlorophyll a, b and total decreased, which indicates However, viruses causing mosaicism, including bean yellow mosaic virus (BYMV), had a clear effect on chlorophyll, which may lead to its destruction, decomposition or prevention of its formation in the plant. This is consistent with what (Tu et al., 1968), stated that infection with the virus reduces the photosynthesis process in the parenchyma tissues of infected leaves, with a smaller size of plastids and a reduction in the numbers of small, medium and large plastids. The inhibition of chlorophyll formation in infected leaves is due to the accumulation of some metabolic compounds such as soluble carbohydrates, which in turn leads to a reversible inhibition of the photosynthesis process. The results are consistent with (Younis et al, 2022).

Table 2. Effect of treatments on the chlorophyll content of fava bean leaves infected with BYMV mosaicism

mg/gm tissue			Treatments
Chlorophyll total	Chlorophyll b	Chlorophyll a	
16.03 c	17.21 c	19.44 d	Comparison Treatment
19.77 b	22.13 b	22.92 c	Mineral Oil
17.13 c	18.08 c	20.85 c	Insecticide
21.9 b	23.81 a	24.84 b	Mineral Oil + Insecticide
25.93 a	30.02 a	34.11 a	Plant Barrier

Numbers with similar letters do not differ significantly at the probability level of 5%

2-The Amount of Nutrients for the Leaves of Fava Beans Infected with BYMV

The statistical analysis in Table (3) shows that there are significant differences among the treatments on the amount of nutrients for the leaves of fava beans. This effect was towards a decrease in calcium, potassium and sodium and an increase in nitrogen for infected plants, whereby potassium reached (1.81) mg, sodium (1.843) mg, calcium (1.732) mg and nitrogen (1.567) mg, respectively, compared to the plant barrier treatment and the

mineral oil treatment with insecticide (Ilić et al., 2019). As for the number of elements for the plant barrier treatments and the mineral oil treatment with insecticide, there were no significant differences for the comparison treatment. Thus, these results indicate that the treatments have maintained the concentration of nutrients compared to infected plants, as we notice a decrease in calcium, potassium and sodium, which indicates that the infection affects the leaves, whether they are soft or dry, and reduces them, as viral infections have a clear effect on the deficiency of some mineral elements in the plant, including major elements, when calculated based on the dry weight of the plant. As for the increase in the amount of nitrogen in infected plants compared to other treatments, it may be attributed to the severe activity of the virus in infected plants, especially the multiplication process, as it needs nitrogen to build its nucleic acid and protein, which leads to an increase in the percentage in the infected plants, but this increase was certainly not in the interest of the plant, as its effect may be opposite and negative (Mathews, 1981). The results are consistent with (Younis et al., 2022; Muhammad et al., 2021; Mathews, 1981).

Table 3. The effect of treatments on the amount of nutrients in the leaves of fava beans infected with BYMV mosaic

mg/gm tissue				Treatments
Nitrogen	Calcium	Sodium	Potassium	
1.567 a	1.732 d	1.843 d	1.81 c	Comparison Treatment
1.543 b	1.996 b	1.967 b	1.932 b	Mineral Oil
1.55 b	1.941 d	1.956 c	1.91 b	Insecticide
1.54 c	2.014 a	2.039 a	2.024 a	Mineral Oil + Insecticide
1.9 c	2.096 a	2.043 a	2.032 a	Vegetable Barrier

Numbers with similar letters do not differ significantly at the probability level of 5%

Conclusions Recommendations

Conclusions

1- The highest infection rate with the BYMV virus was recorded in the pre-flowering stage in Al-Huwayjah, while the lowest infection was in Daquq for the 2022 season. As for the 2023 season, the highest infection rate was in the Al-Huwayjah area, while it was the lowest in the Daquq area.

2- The results of the serological diagnosis of the isolate showed that the main cause of mosaic symptoms on the fava bean plant included in the study is the BYMV virus.

3- Regarding the effect of treatments on the chlorophyll content of fava bean leaves, the plant barrier had a clear effect in increasing the amount of chlorophyll or maintaining the amount of chlorophyll A, B and total in fresh fava bean leaves.

4- Regarding the amount of nutrients in fava bean leaves, it was towards a decrease in calcium, potassium and sodium and an increase in nitrogen related to infected plants.

Recommendations

1- Adopting the virus detection method as an appropriate and safe method due to its efficiency.

2- Expanding the study relating to the effect of the studied parameters on other crops that are subjected and sensitive to this disease along with various elements to measure the tolerance of these plant families.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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