

Original Research Article

Dose-Dependent Morphological Inhibition and Genomic Instability in Broad Bean (*Vicia faba* L) Exposed to Sodium Hypochlorite

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Abstract: Residual chlorine resulting from treatment of drinking water may be an unrecognized source of abiotic stress in agrosystems. This study aimed to, using a integrated morphological and RAPD-PCR method, analyse the dose-dependent effects of sodium hypochlorite on morphological performance and genomic stability of broad bean (*Vicia faba* L.). Seeds were treated with five different chlorine levels (0.5, 1, 2.5, 5 and 10 mg l⁻¹) and a control treatment irrigated with river water. Morphological characteristics, such as height of plants, leaf number, branching, flowering and leaf size were recorded while molecular changes were screened by ten RAPD primers. The outcomes indicated that all vegetative growth parameters decreased significantly ($p \leq 0.05$) with increasing concentration of chlorine and severe inhibition was detected at 5 mg/l and sometimes \Rightarrow 10 mg/l whereas low exposure to chlorine (1 mg/l) caused slight stimulation in branching of tomato with no stimulating effect on foliage count as evidence for manifestation of hormesis response. RAPD analysis indicated that significant genomic instability occurred under high dose of chlorine, including new appearance and absent bands of DNA and its variation being 77%-100%. The number of unique RAPD bands also increased with increasing chlorine concentration; indicating that the genotoxic effects were dose-dependent. These results indicate that NaOCl has a dual action in broad bean, i.e., an enhancing effect at low doses and morphological inhibition and genomic instability under high dose. RAPD markers were found to be sensitive in the early detection of genotoxic stress caused by chlorine and it emphasized the necessity of controlling residual chlorine in irrigation water for crop genetic integrity.

Keywords: *Vicia Faba* L, Sodium Hypochlorite, Genotoxicity, RAPD-PCR, Morphological Traits.

INTRODUCTION

The broad bean (*Vicia faba* L.) is one of the leading winter legumes with a large amount of proteins, playing an important role in biological nitrogen fixation and sustainable agriculture. The crop makes a substantial contribution to soil fertility amelioration, reduces dependency on synthetic nitrogen fertilizer and is therefore crucial for food security in semi-arid and developing areas (Rubiales *et al.*, 2021). Despite its agronomic and dietary value, *V. faba* is known to be very sensitive to environmental and chemical injuries that could have detrimental effects on the growth habits, productivity and genetic fidelity of this plant.

More recently, the use of treated surface and ground water for irrigation has increased, which has raised questions about remnant disinfectants from drinking water treatment practices. Sodium hypochlorite is an effective disinfectant, which can remain in the form of residual chlorine in irrigation water and tend to accumulate slowly in soil-plant systems. It has been shown recently that irrigation water with residual concentrations of chlorine which are within the safe level for human consumption may still elicit phytotoxic effects in sensitive crops, depending on exposure level and time (McGehee *et al.*, 2023; McDonald *et al.*, 2021; FAO, 2023). These results underline that irrigation with the same water quality could not be deduced from drinking standards, without taking into account plant isolated responses.

Chlorine as a two-faced player in plants. Chloride ions play an important role in osmotic potential, stomatal regulation and increase in WUE at low concentration. The beneficial impact of supplying chloride on photosynthetic

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performance and plant water relations have been previously reported under certain environmental conditions in a number of recent studies (Franco-Navarro *et al.*, 2021; Lucas *et al.*, 2024; McDonald *et al.*, 2021). However, if the exposure to chlorine is excessive from the exhaustion of Cl-generators, ionic and nutritional balances are disturbed, resulting in chlorosis, growth retardation and lowered biomass accumulation. Legumes, such as *V. faba* are among the most sensitive to high Cl concentrations and can show significant decreases in vegetative growth when grown under restrictive (high) levels of chlorine ([CL]; Bélanger *et al.*, 2024).

At the cellular and molecular level, chlorine-induced stress is also closely related to oxidative stress caused by excess production of reactive oxygen species (ROS). The oxidative stress can damage lipids, proteins and nucleic acid when the production of ROS surpasses the detoxification capacity of antioxidant systems. These processes may cause DNA damage and genomic instability long before the appearance of gross morphological signs (Hasan *et al.*, 2017; Induced Genotoxicity and Oxidative Stress in Plants, 2021). Therefore, only morphological criteria depend on for assessments apparently do not reflect that actual degree of stress caused by chlorine in a plant.

For this reason, molecular marker methods have been increasingly used to complement morphological assessments in stress research. Random Amplified Polymorphic DNA (RAPD-PCR) profile has been suggested as one of the most rapid and low-cost screening techniques for detection of stress-induced genetic variation, where RAPD banding pattern alterations in response to chemical exposure frequently represent genotoxins-mediated DNA damage or even karyotype structural genomic rearrangements (Atienzar & Jha, 2006; Singh *et al.*, 2022). In addition, *Vicia faba* has been broadly employed as a sensitive plant bioassay for testing the toxicity and genotoxicity of environmental contaminants which confirmed the suitability of it in molecular stress investigation (Li *et al.*, 2023).

Therefore, this study aims to explore the concentration-dependent influence of sodium hypochlorite on morphological behaviour and genetic stability in *Vicia faba* incorporated with vegetative growth analysis along with RAPD-PCR profiling. It is suggested that high levels of chlorine lead to the gradual inhibition of vegetative growth and the induction of a genomic instability detectable at the molecular level, while low levels of chlorine might have a positive effect on several morphological traits. This combined approach offers science-based views of the boundary between appropriate and detrimental chlorine levels and facilitates efforts to adopt rational irrigation water regulations for maintaining crop productivity and genetic integrity.

MATERIALS AND METHODS

Plant Material and Experimental Design

Seeds of certified broad bean (*Vicia faba* L.) were purchased from Al-Fallah Agricultural Office (in Tikrit City, Salah Al-Din Province, Iraq). Seeds were surface rinsed with deionized sterilized water to eliminate attached impurities. The research was conducted using a completely randomized design (CRD) with five NaOCl treatments (0.5, 1.0, 2.5, 5.0 and 10 mg L⁻¹), as well as a control treatment irrigated with untreated river water.

Seeds were pre-germinated in the dark for 48 h and then transplanted out into plastic pots filled with a homogenized growth medium. Plants were grown in a greenhouse at College of Science, Tikrit University using the same conditional field throughout all experimental periods. Uniform application of treatments and collection of plant samples one month after planting for morphological and molecular analyses.

Morphological Trait Assessment

Ten randomly selected plants per treatment were evaluated for morphological parameters. The following parameters were recorded:

- Plant height (cm) = from stem base to apical meristem.
- Leaf number: It was counted in plants.
- Number of branches: per plant.
- Flowering: flowering point was yes and no.
- Leaf size (cm): recorded as mean length of full expanded leaves.

For each parameter and treatment, average values were determined.

Sample Collection for Molecular Analysis

Young, fully expanded leaves were collected from apical regions of plants one month after planting. Leaf samples were placed in sterile, labeled bags and immediately transported to the laboratory for genomic DNA extraction.

Genomic DNA Extraction

Genomic DNA was isolated from fresh leaf tissue with a plant genomic DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's protocol. In brief, leaf tissue was ground in liquid N₂ form a fine powder and cell lysis, protein precipitation, DNA binding, washing, elution were made. Agarose gel electrophoresis was used to check DNA quality and integrity, while purity of the DNA was determined spectrophotometrically from A₂₆₀/A₂₈₀ absorbance ratios. DNA samples were kept at -20 °C until further analysis.

RAPD-PCR Amplification

RAPD-PCR analysis was performed using ten random primers (Bioneer, Korea). PCR reactions were prepared using AccuPower® PCR PreMix (Bioneer, Korea) in a total reaction volume of 20 µL containing genomic DNA, primer, and nuclease-free water. Amplifications were carried out in a thermal cycler under the conditions summarized in Table 1.

PCR products were separated by electrophoresis on 2% agarose gels, stained, and visualized under ultraviolet light. Band sizes were estimated by comparison with a standard DNA ladder.

Table 1: RAPD-PCR Thermal Cycling Program

STEP NAME	TEMPERATURE (°C)	NUMBER OF CYCLES	STEP DURATION
INITIAL DENATURATION	94	1	4 MINUTES
DENATURATION	93	40	45 SECONDS
PRIMER ANNEALING	36	40	1 MINUTE
EXTENSION	72	40	1.5 MINUTES
FINAL EXTENSION	72	1	10 MINUTES

RAPD Data Scoring and Analysis

The clear and distinguishable RAPD bands were manually scored as presence (1) or absence (0) to create a binary table. Only bands that were continuously over-expressed were selected for analysis. Primer efficiency, discriminatory power and percentage of polymorphism were estimated based on standard formulas provided in earlier reports (Grudman *et al.*, 1995).

Statistical Analysis

The morphological traits were subjected to analysis of variance (ANOVA) statistics using a completely randomized design (CRD). Means were compared among treatments using Duncan's Multiple Range Test at $P \leq 0.05$. Statistical analyses were performed with the SAS statistical analysis system (SAS, version 2001). Pearson correlation coefficient was estimated, and the characteristics were correlated (Al-Rawi and Khalaf Allah, 2000).

RESULTS

Effects of Sodium Hypochlorite on Morphological Traits

Dose-dependent responses of *Vicia faba* plants to increasing sodium hypochlorite concentrations were observed based on all the morphological characters examined (Table 2). Body Figure 1: Representative visual comparison of plant growth and vigor under different treatments.

The highest mean plant height was reached at 0.5 and 1.0 mg L⁻¹, with a sharp decrease ($P \leq 0.05$) in relation to higher levels of chlorine.

Table 2: Effects of different chlorine concentrations on morphological traits of broad bean (*Vicia faba* L)

NO.	TREATMENT	PLANT HEIGHT (CM)	NUMBER OF BRANCHES	NUMBER OF LEAVES	FLOWERING	NUMBER OF PODS	LEAF SIZE (CM)
1	F1	15	2	18	ABSENT	ABSENT	4.5
2	F2	15	10	21	PRESENT (1)	ABSENT	2.2
3	F3	12	2	14	PRESENT (1)	ABSENT	3.3
4	F4	11	3	14	PRESENT (1)	ABSENT	3.4
5	F5	11	3	2	ABSENT	ABSENT	1.9



Figure 1: Growth responses of broad bean (*Vicia faba* L.) to sodium hypochlorite treatments

Maximum mean plant height (15 cm) was attained at 0.5 and 1.0 mg L⁻¹, with a subsequent significant decrease ($P \leq 0.05$) at 2.5 mg L⁻¹ and above to least values observed at 5.0 and 10.0 mg L⁻¹ respectively [Table-I]. Trends in leaf number and the size of leaves were also similar, being highest at 1.0 mg L⁻¹ with marked decline under high chlorine treatments.

Branch response varied between treatments and was most expressed at 1.0 mg L⁻¹ (ten branches per plant) indicating a stimulatory effect early on in chlorine challenge. Conversely, branching of plants treated with 5.0 and 10.0 mg L⁻¹ was significantly lower, suggesting an inhibitory response to elevated chlorine levels.

Flowering did not occur in the lowest treatments (0.5 and 1.0 mg L⁻¹), with reduced flowering at intermediate concentrations (2.5 and 5.0 mg L⁻¹). No podding was observed in any treatment over the duration of the experiment, suggesting that sodium hypochlorite exposure had an adverse effect on reproductive development regardless of concentration.

As a whole, the morphological data showed a dose-dependent inhibitory effect of sodium hypochlorite on vegetative growth, and there were few indications that narrow concentrations around 1.0 mg NaCl L⁻¹ evoked no or stimulatory responses.

Genomic DNA Quality and RAPD-PCR Profiles

RAPD-PCR amplification generated clear and reproducible banding patterns across all sodium hypochlorite treatments and the control. Representative RAPD profiles obtained using selected primers are shown in Figure 2.

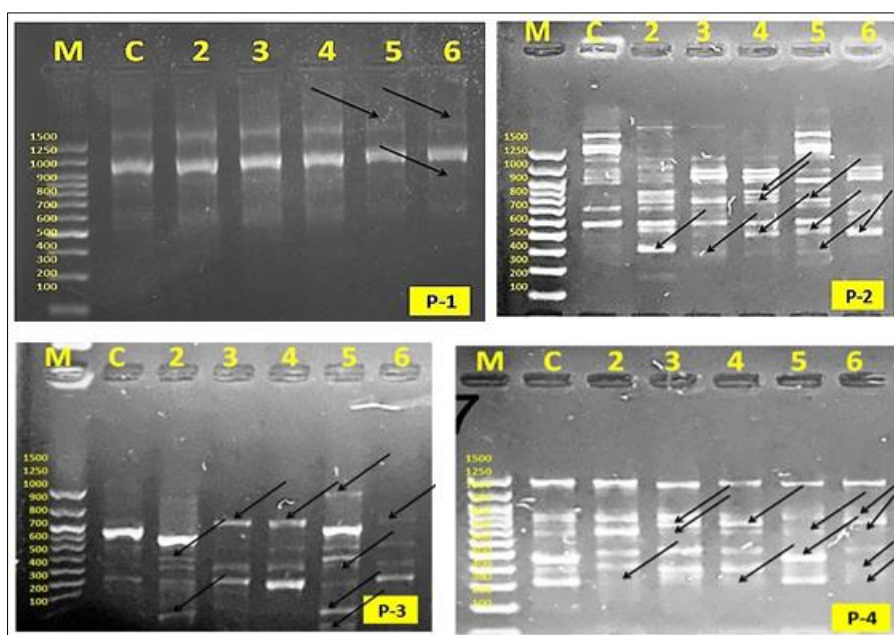


Figure 2: RAPD-PCR banding patterns of broad bean (*Vicia faba* L.) plants exposed to different sodium hypochlorite concentrations

Chlorine-treated plants exhibited distinct alterations in banding patterns compared with the control, including the appearance and disappearance of DNA bands. These alterations became more pronounced with increasing sodium hypochlorite concentration, indicating progressive genomic instability.

RAPD Polymorphism and Primer Efficiency

The efficiency and discriminating power of the RAPD primers were differential. The percentages of polymorphism varied from 77% (both OPC-01 and OPB-07) to 100% (OPC-05, OPQ-15 and OPJ-12), indicating that these primers were highly sensitive for the detection of chlorine-induced genetic variation.

The unique RAPD bands were increased by increasing chlorine (Table 3). There were no specific bands in the control treatment to suggest genomic stability in conditions of no treatment. On the other hand, unique bands numbers in treated plants significantly enhanced with increasing doses and its highest value was recorded at 10.0 mg L⁻¹ sodium hypochlorite. This pattern validates a dose-dependent rise of genetic aberrations related to chlorine monitoring.

Table 3: RAPD polymorphic bands generated by different primers in broad bean (*Vicia faba* L.) plants treated with sodium hypochlorite

NO.	PRIMER NAME	MOLECULAR SIZE (BP)	MC	M1	M2	M3	M4	M5	TOTAL POLYMORPHIC BANDS
1	OPC-05	100–800	–	–	1	–	–	1	3
2	OPQ-15	450–1000	–	–	–	–	1	1	2
3	OPG-15	300–1100	–	–	–	1	–	–	2
4	OPJ-12	475–1500	–	–	1	–	–	–	1
5	OPJ-14	425–1800	–	–	–	–	1	–	1
6	OPG-11	425–1600	–	–	1	–	1	–	2
7	OPW-08	175–950	–	–	1	–	–	–	1
8	OPW-09	200–2500	–	–	–	1	1	–	2
TOTAL	—	—	0	0	4	2	4	2	14

Statistical Analysis of Morphological and Molecular Responses

However, differences between the treatments were significant ($P \leq 0.05$) for most of the morphological traits as indicated by statistical analysis. Batches high in chlorine (5.0 and 10.0 mg L⁻¹) differed significantly from the control and low-concentration treatments, reflecting a pulping inhibition effect. By contrast, intermediate treatments exhibited a range of responses, suggesting the existence of a threshold for tolerance that when surpassed triggers strong stress effects.

At the molecular level, high percentage of polymorphism and number of unique RAPD bands at higher Cl concentrations corroborated the morphological results indicating that phenotypic inhibition was coincided with measurable genomic instability.

DISCUSSION

[Effects of hypochlorite sodium on morphomethrical and genomic stability of broad bean (*Vicia faba* L.)]. The decrease of plant height, leaf number, size and branching observed at elevated chlorine levels indicates that residual chlorine is clearly an important abiotic stress at higher concentrations. The growth inhibition conditions for legumes and other crops are comparable when plants are exposed to chlorine containing irrigation water, or subjected to a chloride stress, which have show nitrate imbalance and photosynthetic impairment due to excessive chlorine (Hasan *et al.*, 2017; Bélanger *et al.*, 2024; McGehee *et al.*, 2023). Strikingly, low chlorine exposure (especially 1.0 mg L⁻¹) caused the partial preservation or activation of some morphological characteristics like branching and leaf growth. This finding is in agreement with the idea of the hormetic or dual role effect of chlorine, since low concentrations exert stimulating activity on physiological regulation and higher concentrations are toxic towards cells. Previous studies demonstrated the role of chloride as a beneficial micronutrient in osmotic regulation and cadmium (Cd) tolerance, stomatal control, and enhanced water-use efficiency when it is at an optimal range (Franco-Navarro *et al.*, 2021; Wege *et al.*, 2021; Lucas *et al.*, 2024). Nevertheless, this positive effect seems to be restricted to a very narrow range of concentration above which growth inhibition is prevailing.

The lack of pod formation at all levels of chlorine indicates that reproductive growth in *V. faba* may be highly sensitive to chlorine dose. Reproductive characteristics are generally more susceptible to environmental stress than vegetative growth because stress-induced alterations in assimilate distribution and hormone economy can interfere with the process of flowering initiation and fruit set. The similar decrease in reproductive performance found due to chemicals and salinity as stress effect on α AI could also be observed in broad bean, peas and other legumes (Abdul Qados, 2011; Metwali *et al.*, 2015).

Molecular data as obtained by RAPD-PCR showed substantial changes in banding patterns of the chlorinetreated plants as compared to control ones. The emergence of new bands and the fading of old bands while increasing the chlorine concentration suggests genomic instability. These RAPD profile modifications are commonly interpreted as a sign of DNA damage, mutation or structural reshuffling at the primer binding sites resulting from environmental stress factors (Atienzar and Jha, 2006; Singh and Kumar, 2019; Singh *et al.*, 2022). In addition, the counts of unique RAPD bands increased progressively at higher chlorine levels which also confirm the genotoxicity of sodium hypochlorite to bacteria under extended exposure.

The molecular data are in good agreement with previous studies that have also revealed stress-induced genotoxicity of *Vicia faba* under salinity, heavy metals and chemicals treatments; here, the increased polymorphism and decreased genetic similarity between RAPD markers were identified (Metwali *et al.*, 2015; Abdalla *et al.*, 2018). In addition, *V. faba* is a widely known sensitive bioindicator for cytotoxic and genotoxic tests conferring validity to the RAPD reactions obtained (Li *et al.*, 2023).

Oxidative stress may play an important role in chlorine-triggered genomic instability. Overexposure to chlorine might increase the generation of reactive oxygen species (ROS), resulting in oxidative damage of cellular macromolecules, such as nucleic acids. If the wins of free radicals, that cannot be countered by antioxidant defense systems, reach harmful levels (obviously lower than lethal ones), then DNA-repair-deficient hold-up in DNA lesions and chromosomal alterations can accumulate till becoming visible molecular changes but still before lethal morphological lesion become evident (Hasan *et al.*, 2017; Induced Genotoxicity and Oxidative Stress in Plants, 2021). The good agreement of morphological inhibition and RAPD-detected genomic alterations in the current study is consistent with this mechanistic explanation.

Taken as a whole, the combination of morphological and molecular analyses gave a broad view on chlorine stress impact in *Vaba*. The strong correlation between inhibition of growth and increased RAPD polymorphism indicates that morphological responses alone are not useful for detecting host genotoxic effects at early stages, while molecular markers can provide sensitive monitoring tools for genotoxic stress. Such conclusions have been also reached in recent works regarding the usefulness of RAPD markers for rapid screening methodologies aimed at assessing plant responses to environmental stresses (D'Angeli *et al.*, 2024).

CONCLUSIONS

In the present work, it is clear from our research that sodium hypochlorite has dose-dependent effects on morphological characters of broad bean (*Vicia faba* L.) as well as genomic stability. Mild exposure to chlorine (1.0 mg L⁻¹) resulted in no significant or mild stimulation of some vegetative traits and a concentration (≥2.5 mg L⁻¹)-dependent growth inhibition, phenoloxidase activity and reproductive damage.

Genetic diversity and number of unique DNA bands increased with increase in concentration of chlorine suggesting gradual destabilisation of genome and induction genotoxic stress. These molecular variations correlated with the observed morphological inhibition, indicating that chlorine-induced stress impairs also phenotypic and genomic stability.

The combination of morphological evaluation and RAPD markers was successful in discriminating early stress responses which may not be fully expressed at the level of visible growth. The detected RAPD polymorphisms might be used as potential molecular markers for chlorine sensitivity in *V. faba*.

Collectively, the results underscore the need to control residual chlorine levels in irrigation water because higher concentration beyond a critical narrow window can impair both plant morphological traits and genetic stability. These findings are of great importance for the management of irrigation water and VM strategies in protecting crop productivity and genetic purity under growing use of treated water.

REFERENCES

- Abdalla, M. M., El-Sayed, A. A., & Mohamed, M. A. (2018). Molecular characterization of salt tolerance in faba bean (*Vicia faba* L.) using RAPD markers. *Journal of Genetic Engineering and Biotechnology*, 16(1), 77–86.
- Abdul Qados, A. M. S. (2011). Effect of salt stress on plant growth and metabolism of bean plant (*Vicia faba* L.). *Journal of the Saudi Society of Agricultural Sciences*, 10(1), 7–15. <https://doi.org/10.1016/j.jssas.2010.06.002>
- Atienzar, F. A., & Jha, A. N. (2006). The random amplified polymorphic DNA (RAPD) assay and its application in genotoxicity studies. *Mutation Research/Reviews in Mutation Research*, 613(2–3), 76–102. <https://doi.org/10.1016/j.mrrev.2006.06.001>
- Bélanger, J. G., Gélinas, J., & Tremblay, G. F. (2024). Chloride toxicity and mitigation strategies in legumes: Physiological and agronomic perspectives. *Plant Methods*, 20(1), 79. <https://doi.org/10.1186/s13007-024-01069-4>

- D'Angeli, S., Altamura, M. M., & Falasca, G. (2024). Plant responses to abiotic stress: Molecular markers as early indicators. *Plant Stress*, 6, 100105. <https://doi.org/10.1016/j.stress.2023.100105>
- Franco-Navarro, J. D., Díaz-Rueda, P., Rivero, R. M., & Brumós, J. (2021). Chloride nutrition improves water use efficiency and photosynthetic performance in plants. *Journal of Experimental Botany*, 72(9), 3444–3459. <https://doi.org/10.1093/jxb/erab109>
- Hasan, M. K., Cheng, Y., Kanwar, M. K., Chu, X. Y., Ahammed, G. J., & Qi, Z. Y. (2017). Responses of plant proteins to heavy metal stress—A review. *Frontiers in Plant Science*, 8, 1492. <https://doi.org/10.3389/fpls.2017.01492>
- Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfiqar, F., et al. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress. *Antioxidants*, 9(8), 681. <https://doi.org/10.3390/antiox9080681>
- Li, Y., Zhang, H., & Wang, X. (2023). Application of *Vicia faba* bioassay for cytotoxicity and genotoxicity assessment of environmental contaminants. *Environmental Toxicology and Chemistry*, 42(5), 1120–1130. <https://doi.org/10.1002/etc.5612>
- Lucas, M., Etienne, P., & Lejay, L. (2024). Chloride as a beneficial macronutrient: Physiological and agronomic implications. *Plant Physiology and Biochemistry*, 201, 108018. <https://doi.org/10.1016/j.plaphy.2024.108018>
- McDonald, G. K., Tavakkoli, E., & Rengasamy, P. (2021). Salinity, sodicity and chloride toxicity in crop plants. *Plant and Soil*, 466, 1–15. <https://doi.org/10.1007/s11104-021-05028-3>
- McGehee, C. S., Poole, R. T., & Lieth, J. H. (2023). Phytotoxicity of residual chlorine in irrigation water used for horticultural crops. *HortTechnology*, 33(2), 145–152. <https://doi.org/10.21273/HORTTECH05031-22>
- Metwali, E. M. R., Abdelmoneim, T. S., & Bakheit, M. A. (2015). Molecular diversity of faba bean (*Vicia faba* L.) genotypes under salinity stress using RAPD markers. *International Journal of Agriculture and Biology*, 17(1), 47–54.
- Rubiales, D., Flores, F., Emeran, A. A., Kharrat, M., Amri, M., Rojas-Molina, M. M., & Sillero, J. C. (2021). Legume breeding for the agroecological transition of global agri-food systems: A European perspective. *Frontiers in Plant Science*, 12, 782574. <https://doi.org/10.3389/fpls.2021.782574>
- Singh, S., Prasad, S. M., & Sharma, S. (2022). Molecular markers as tools for assessment of genotoxic stress in plants. *Environmental and Experimental Botany*, 198, 104867. <https://doi.org/10.1016/j.envexpbot.2022.104867>
- Wege, S.-M., Khan, G. A., Jung, J.-Y., Vogiatzaki, E., Pradervand, S., Aller, I., ... Poirier, Y. (2021). Chloride: An essential macronutrient and regulator of photosynthesis and water use efficiency in plants. *Journal of Experimental Botany*, 72(9), 3444–3459. <https://doi.org/10.1093/jxb/erab109>