
January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

# Committees: The Second International Conference on Genetic Resources and Biotechnology

Cite as: AIP Conference Proceedings **2462**, 010002 (2022); https://doi.org/10.1063/12.0008934
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# Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24$^{th}$-25$^{th}$ of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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# The effect of $FeSO_4$ concentration on the callus growth of two chili (*Capsicum annuum* L.) varieties

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**Rossa Yunita, Endang Gati Lestari, Iswari S. Dewi, et al.**

# The Effect of $FeSO_4$ Concentration on the Callus Growth of Two Chili (*Capsicum annuum* L.) Varieties

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**Abstract.** *In vitro* selection is a method that can produce varieties that are tolerant to abiotic stresses such as Fe poisoning. $FeSO_4$ is a compound that can be used as an *in vitro* selection agent for resistance to Fe toxicity. Each genotype has a different response to $FeSO_4$. For this reason, it is necessary to determine the optimal concentration for each genotype to be selected so that the selection results are more accurate. This study aimed to observe the response of callus growth to Fe toxicity and to obtain the lethal concentration of 50 ($LC_{50}$) $FeSO_4$ value for callus of two chili varieties Prima Agrihorti and Rabani Agrihorti. This study used a complete factorial design with the first factor was variety and the second factor was the concentration of $FeSO_4$ (0, 54, 108, 162, and 215 mg/l). The number of repetitions was 10 times. The observed parameters were the percentage of browning callus, callus diameter, and callus performance. To determine the $LC_{50}$ value, the curve analysis program was used. The results showed the interaction between varieties and the concentration of $FeSO_4$ has not significant effect, but when the $FeSO_4$ concentration increase, the growth was inhibited, shown as smaller diameter and the callus turned to brown. The $LC_{50}$ $FeSO_4$ value in the variety Prima Agrihorti was 130.341 mg/l, while that of Rabani Agrihorti was 115.511 mg/l. These values can be used for *in vitro* selection of callus of chili varieties Prima Agrihorti and Rabani Agrihorti.

## INTRODUCTION

Iron is a major constraint on new land in tidal areas or in basin areas. Iron poisoning is caused by large concentrations of Fe in the soil solution. Old soils, such as Oxisols, red Latosol, red Podzolic, or acid sulphate soils, are inundated with $Fe^{3+}$. $Fe^{3+}$ is immobile which will reduce to $Fe^{2+}$ which is mobile. At certain concentrations it is toxic to plants [1]. At levels more than 300 ppm in tissue, the presence of Fe can cause poisoning in plants [2].

$Fe^{2+}$ which passes through the Casparian strip in the endodermis layer will spread to all parts of the plant roots. Fe will accumulate in the roots and be translocated through the xylem to the leaves. Excess $Fe^{2+}$ in leaves causes the formation of several types of active oxygen, such as superoxide and peroxide, with high concentrations, excessive accumulation of Fe. This process results in an oxidation reaction of phenol to polyphenol which will accumulate to form bronzing on the leaves [3].

The formation of plaque due to $Fe^{2+}$ oxidation on the root surface is one of the tolerance mechanisms for Fe. In addition to the tolerance mechanism in the roots, the tolerance mechanism for Fe poisoning in leaf tissue, leaf cells, $Fe^{2+}$ will catalyze the formation of various types of active oxygen and radical compounds [4]. The mechanism for avoiding iron poisoning in rice plants can also be done by increasing the pH of the rhizosphere, by releasing OH ions from the roots [5–7].

Iron is an important element in the metabolism of plant growth. However, large amounts of Fe can have a negative effect on plant growth because inorganic iron has low solubility and the physiological pH is very reactive in the presence of oxygen, causing toxic hydroxyl radicals [8].

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Chili is one of the important horticultural commodities in Indonesia. In Indonesia, there are two types of chili, namely large chili (*Capsicum annuum*) and cayenne pepper (*C. frutescens*) [9]. The demand of these varieties is increasing, both for household needs and for industrial scale. Available land for chili plants is swampland with Fe stress. Large amounts of iron will have a bad effect on chili plants, where growth will be stunted and reduce chili production because large amounts of Fe will interfere with plant metabolism [10].

One of the methods to obtain high Fe-tolerant varieties is mutation breeding combined with *in vitro* selection. To select cell population, the selection agent $FeSO_4$ was used to screen cells that were highly tolerant of Fe. The purpose of this study was to observe the response of callus growth to Fe toxicity and to obtain the lethal concentration of 50 ($LC_{50}$) $FeSO_4$ value for callus of two chili varieties Prima Agrihorti and Rabani Agrihorti.

## MATERIALS AND METHODS

This research was conducted at the Tissue Culture Laboratory of Cell and Tissue Biology Division, Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIGRAD) from June to December 2020. The plant material used as explants was callus from *in vitro* leaves of Prima Agrihorti and Rabani Agrihorti varieties. Callus induction used MS medium added with 2,4-D 3 mg/l from leaves of chili varieties. Primary callus aged 4 weeks after planting were sub-cultured in MS medium containing $FeSO_4$ at several concentration levels, namely 0, 54, 108, 162, and 215 mg/l. Each treatment in the study consisted of 10 replications, each replication consisted of 10 explants.

The variables observed were callus diameter, callus weight 4 weeks after planting, the percentage of brown callus and callus performance. Data were analyzed to determine lethal concentration. $LC_{50}$ determination, using best curve analysis to get the best equation. The incubation period of callus in selection media containing $FeSO_4$ was 4 weeks.

## RESULTS AND DISCUSSIONS

This study used callus derived from chili leaves. The chili leaves were cultured on 2.4-D 3 mg/l medium and within four weeks an embriogenic callus was produced. From several research results, it was found that 2,4-D was very effective in producing embryogenic callus in chilies [11]. The callus produced is crumbly, yellowish-white in color, and has a globular structure.

### Effect of $FeSO_4$ on Changes in Callus Fresh Weight

Increasing the concentration of $FeSO_4$ in the medium affected the change in callus fresh weight. Fig. 1 showed that increasing the concentration of $FeSO_4$ will decrease the callus fresh weight. This indicates that the addition of $FeSO_4$ in the medium will reduce callus growth.

The 54 mg/l $FeSO_4$ treatment resulted in a decrease in the change in fresh weight of callus of Prima Agrihorti and Rabani Agrihorti which was not significantly different to the control. Increasing the concentration of $FeSO_4$ to 108–215 mg/l resulted in a significant decrease in the change in callus fresh weight (Fig. 1). This shows that the treatment resulted in damage to cells so that they could not grow properly. The movement of iron between cells occurs due to the catalystation of the formation of reactive oxygen species (ROS), such as superoxide, hydroxyl radicals, and $H_2O_2$ through the Haber-Weiss reaction [12]. The reaction is increasing with the increasing concentration of $FeSO_4$ in the media. This increase in iron results in the formation of free radicals that damage cells, such as feryl radicals which together with fatty acids form fatty acid peroxide [13].

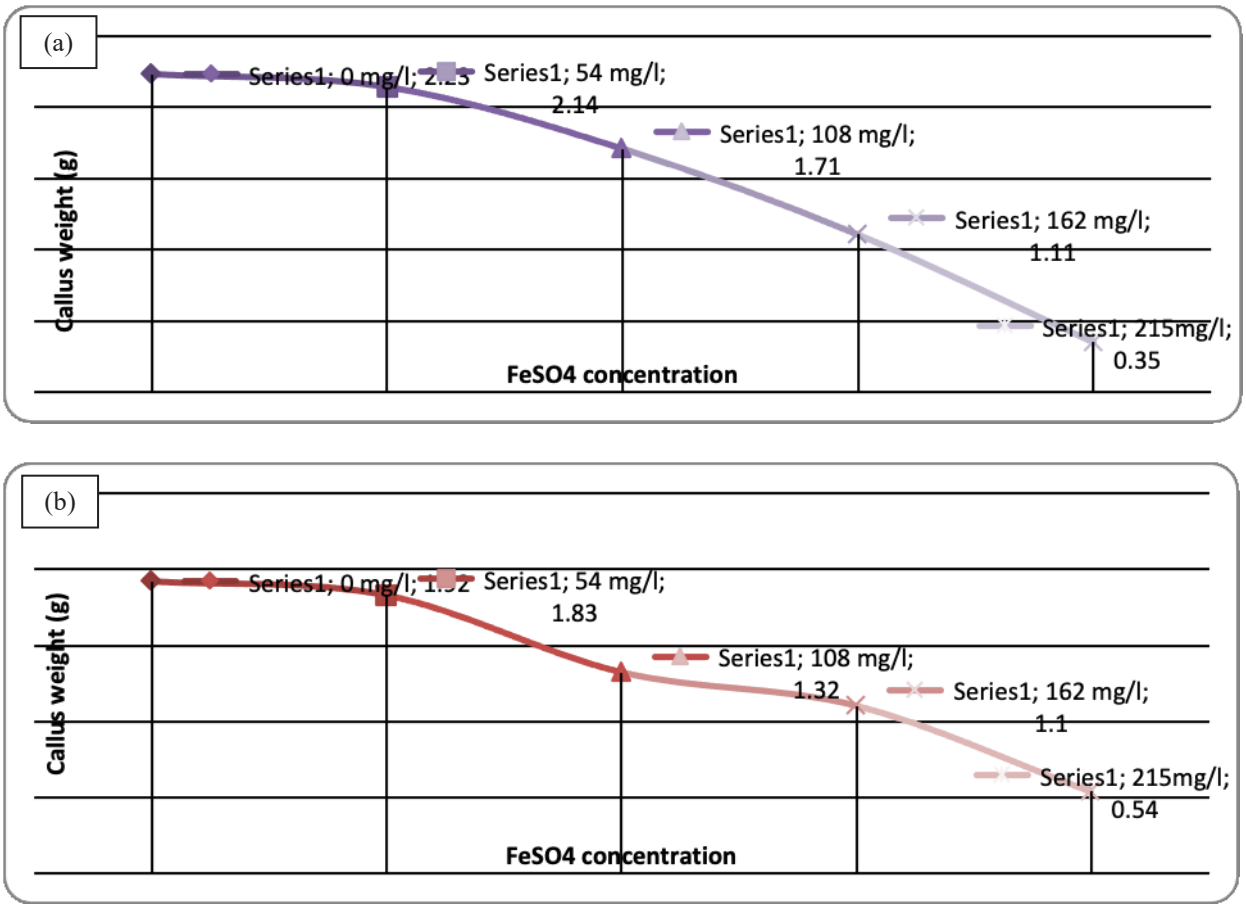


**FIGURE 1.** Effect of $FeSO_4$ concentration on callus diameter of Prima Agrihorti (a) and Rabani Agrihorti (b) varieties at 4 weeks after treatment.

## Effect of $FeSO_4$ on Callus Diameter

Callus diameter was observed at 4 weeks after treatment because the average callus grew the most compared to the previous weeks. Based on Fig. 2, it can be seen that the concentration of $FeSO_4$ has an effect on the increase in callus diameter, where the Prima Agrihorti and Rabani Agrihorti varieties have an average increase in callus diameter getting smaller with the increasing concentration of $FeSO_4$. In Prima Agrihorti variety, the decrease in callus diameter was quite large at 108 mg/l treatment. Whereas for the Rabani Agrihorti variety, $FeSO_4$ 54 mg/l treatment resulted in a decrease in the diameter of the callus which was smaller in size than the control.

High iron concentration inhibited callus growth, where the callus diameter was lower than the control. This showed that $FeSO_4$ compound can be used as a selecting agent for resistance to Fe poisoning. This developed *in vitro* selection method can be used to select mutant callus as an early stage in the selection activity which will be useful for producing varieties tolerant to iron toxicity. Damage to cells so that their growth is inhibited due to inhibited enzyme activity. For tolerant plant callus, it will be able to increase enzyme activity to prevent cell damage due to iron poisoning [14].

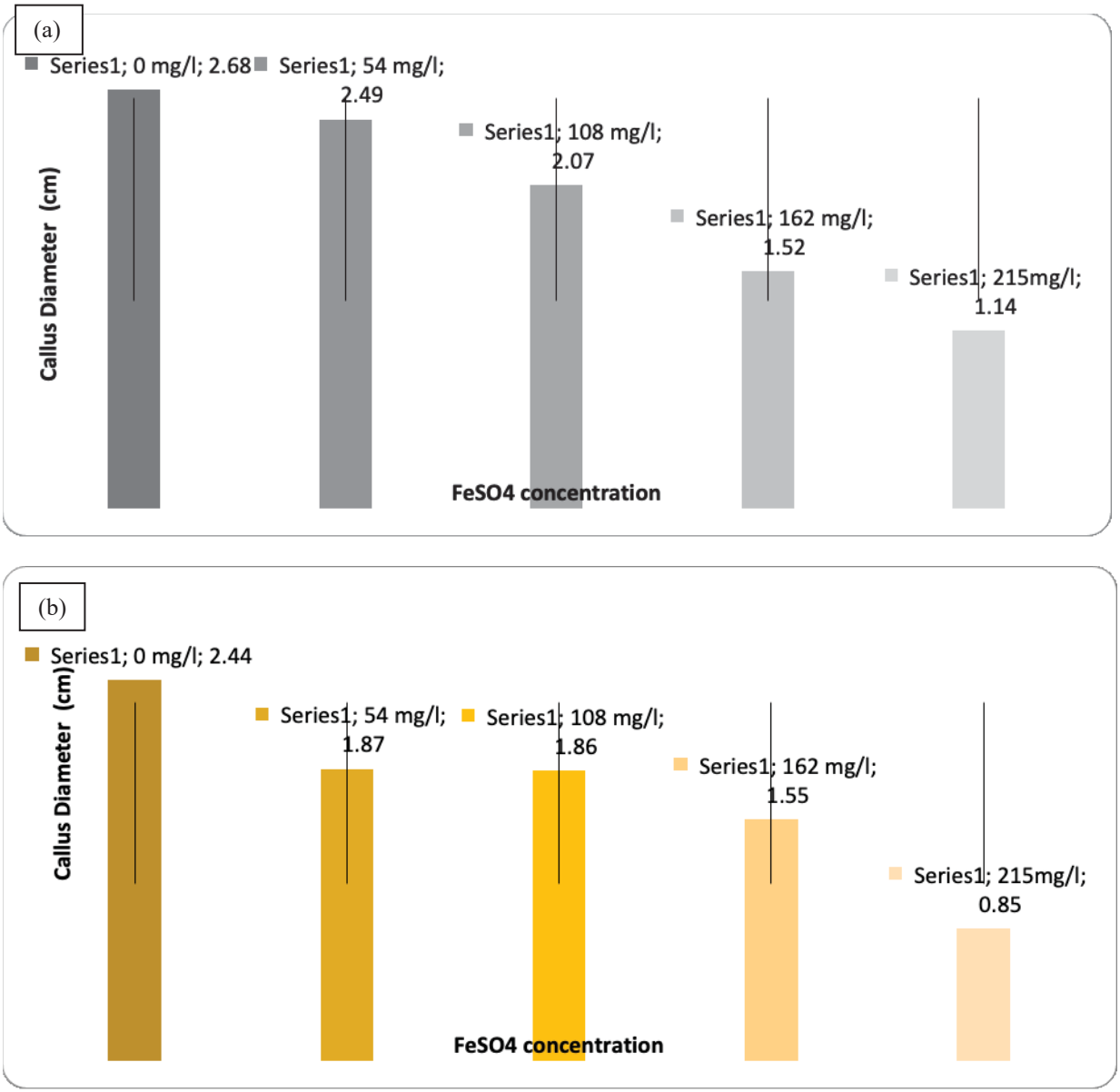


**FIGURE 2.** Effect of $FeSO_4$ concentration on callus weight gain of Prima Agrihorti (a) and Rabani Agrihorti (b) varieties at 4 weeks after treatment.

## Determination of $LC_{50}$ $FeSO_4$ on Callus of Chili Varieties

In Table 1, it can be observed that an increase $FeSO_4$ concentration caused high level of callus browning. The percentage of brown callus was observed after 4 weeks of culture.

In Table 1, it can be seen that an increase in the concentration of $FeSO_4$ resulted in an increase in the percentage of brown callus starting at 54 mg/l treatment. For chili callus, the application of 162 mg/l $FeSO_4$ on the culture media resulted in a callus that began to brown more than 60% in both chili varieties Prima Agrihorti and Rabani Agrihorti.

The results of the best-fit curve analysis program to determine the $LC_{50}$ $FeSO_4$ to chili callus, resulted in the best model equation obtained based on the number of brown calluses (Fig. 3). The value of $LC_{50}$ $FeSO_4$ in the variety Prima Agrihorti was 130.341 mg/l, while that in Rabani Agrihorti was 115.511 mg/l. This indicates that Prima Agrihorti are more tolerant to $FeSO_4$ than Rabani Agrihorti.

**TABLE 1.** Effect of $FeSO_4$ radiation on callus growth of two chili varieties.

| Treatment (mg/l) | Percentage of brown callus (%) | | Callus performance | |
|---|---|---|---|---|
| | Prima Agrihorti | Rabani Agrihorti | Prima Agrihorti | Rabani Agrihorti |
| 0 | 3.0±2.58 | 8.0±3.49 | White | White |
| 54 | 20.0±2.35 | 23.0±6.32 | White | Yellowish white |
| 108 | 34.0±10.75 | 50.0±8.16 | Yellowish white | Yellowish white |
| 162 | 76.5±7.42 | 69.0±5.16 | Brownish yellow | Brownish yellow |
| 215 | 98.2±2.39 | 98.5±2.42 | Brown | Brown |

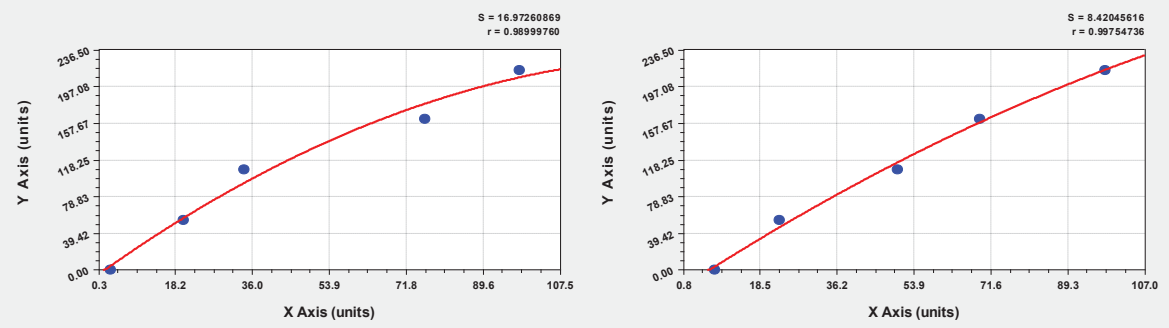


**FIGURE 3.** Result of curve analysis of $LC_{50}$ in callus of chili varieties Prima Agrihorti (left) and Rabani Agrihorti (right).

## CONCLUSION

The conclusion of this study was that the increase in $FeSO_4$ concentration in callus selection medium resulted in a decrease in callus diameter and fresh weight. Based on the results of curvid analysis, the percentage of brown callus resulted in the $LC_{50}$ $FeSO_4$ value of the varieties Prima Agrihorti was 130.341 mg/l and Rabani Agrihorti was 115.511 mg/l. $LC_{50}$ $FeSO_4$ can be applied to increase the genetic diversity of chili varieties Prima Agrihorti and Rabani Agrihorti.

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