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Study of mycoflora associated with seeds of Grass pea (*Lathyrus sativus* L.) in East Azerbaijan

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Introduction

Grass pea (*Lathyrus sativus* L.) is an economically important annual feed and food grain legume in both Asian and African developing countries (Dixit *et al*., 2016). The plant is a short, bushy diploid annuals (2n=14), with blue, pink, or white flowers and green seedpods. Their seedpods are curved and flat and contain 3 to 5 white or grey-brown seeds (Kumar *et al*., 2013). In 2019, the global grass pea acreage was 1.5 million hectares and production was 1.2 million tons. Fungal diseases are among the main factors constraining high grass pea productivity worldwide. Infected seeds by seed-borne fungi and associated with seeds significantly effects on germination and vigor indices and also reduces quality seed and seedling health. Seed health plays an important role in increasing the quantity and quality of yield. Seed health refers specifically to the severity and incidence of pathogens in seeds which play a vital role in carrying pathogens (Mahapatra *et al*., 2019). Fungal pathogens are some of the most important living factors affecting seed health that may be internally associated with seeds or externally seed-borne.

Materials and methods

In order to evaluation of status of fungal infected seeds in grass pea from field of East Azerbaijan (Figure 1) were sampled according to the International Rules for Seed Testing (ISTA) and were transferred to the National Seed Health Laboratory. 400 seeds of each sample were isolated washing with water and the surface was sterilized with 1% sodium hypochlorite solution. Than they were placed on Potato Dextrose Agar (PDA) medium with alternating 12 hours light and 12 hours darkness at 25 ± 1 ˚C (Figure 2). Purification of fungal colonies was performed using single spore and hyphal tip methods on 2% water agar medium. The morphological features (Barnett & Hunter, 1998) and molecular data (one of the marker genomic regions including ITS-rDNA, *Tef1-α*, Gapdh or *Rpb2* based on the studied fungal genus) were examined and the species were identified (Khaledi *et al*., 2024). Further confirmation of the identification was obtained by molecular characterization in which genomic DNA was extracted using DNA extraction kit (Genomic DNA isolation kit IV; DENA Zist Asia, Iran) according to the manufacturer's instructions.

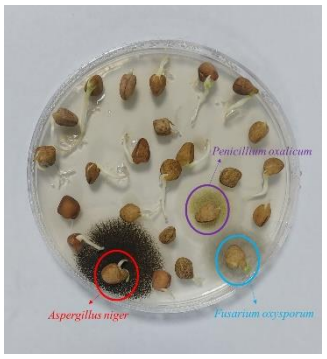

Figure 2: Agar plate method for the detection of seed-borne fungal pathogens of Grass pea (*Lathyrus sativus* L.)

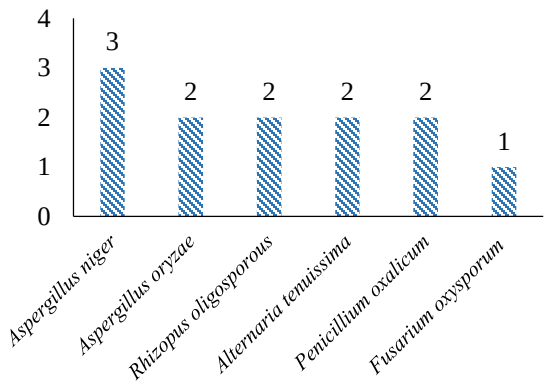

Figure 3: The abundance of seed-borne fungal isolated from Grass pea seeds (*Lathyrus sativus* L.)

Results and Discussion

A total of 12 isolates belonging to five fungal genera were obtained. Based on a combination of morphological features and sequencing data, three isolates belonging to the *Aspergillus niger*, two isolates belonging to the *A. oryzae*, *Rhizopus oligosporous*, *Alternaria tenuissima*, and *Penicillium oxalicum*, one isolate belonging to *Fusarium oxysporum* were identified (Figure 1). Similar results of morphological and molecular identification of fungal species were in accordance with the reports of Sampaio *et al*. (2021) and Buta *et al*. (2020). The *Lathyrus* gene pool offers a source of resistance to important legume diseases caused by fungi, such as ascochyta blight, downy and powdery mildew (Lambein *et al*., 2019). The genera *Aspergillus* (45.4%) and *Fusarium* (0.9%) had the highest and lowest frequency, respectively. The highest frequency of isolated fungi in the seed samples was related to the *A. niger*. Since the species of this genus include pathogens and saprophytes, in order to understand the correct relationship between this isolate and the host plant, pathogenicity tests of the isolate obtained on grass pea are under consideration.

Figure 1: Grass pea (*Lathyrus sativus* L.) seed sample collected from field of East Azerbaijan province of Iran

Conclusion

The results of this study can be used to complete the basic information for identifying of seed-borne fungi of grass pea and help to writing the national seed health standard. This is the first report on isolation and identification of fungal associated with seed of grass pea in Iran.

Keywords

Forage plant, Morphological, Molecular, Seed health.

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