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Screening for resistance of grape varieties to powdery mildew (*Erysiphe necator*) disease

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ABSTRACT

This study was conducted to evaluate the susceptibility of grapevine varieties to powdery mildew. Powdery mildew is a disease caused by a fungal, *Erysiphe necator*, and an obligate parasite of grapevine (*Vitis vinifera L.*). Powdery mildew causes drastic yield losses of 50 to 70%. Commercial grapevines grown in producing countries are susceptible to powdery mildew. Use of fungicides to control the disease is expensive and not environmentally friendly. Therefore, use of grapevine resistant varieties to powdery mildew is cost-effective control method. In this study, ten varieties (Black rose, Regina, Queen of Vineyards, Alphonse lavallee, Makutupora red, Chancellor, Halili belyji, Syrah, Ruby seedless and Makutupora white) were screened for resistance to powdery mildew, using artificial inoculation of spore suspension and dry inoculums. Infected grape leaves were sampled from the field and grounded to obtain powder which was used as dry inoculum. The spore suspension inoculum was made by mixing powder with sterilized distilled water. The inoculation was done in two blocks with concentration of 2×10^5 spore/ml. Disease severity was evaluated based on a scale of 0 – 5; 0, means immune and 5, high level of disease severity. Results showed significant difference ($P < 0.05$) of disease among grapevine varieties evaluated. It was found that 11.1% were resistant, 33.4% tolerant and 55% susceptible to disease. Grapevine variety Chancellor showed the highest level of resistance, and Black rose the most susceptible. The study demonstrates the effectiveness of using inoculation methods in screening resistance to powdery mildew.

Indexing terms/Keywords

Erysiphe necator; Grapevine; Powdery mildew; Resistance; Severity.

Academic Discipline And Sub-Disciplines

The relevant academic disciplines for this journal is based on plant pathology studies

SUBJECT CLASSIFICATION

The subject is Agriculture classification

TYPE (METHOD/APPROACH)

The research type is experiment; laboratory analysis for inoculum and field experiment evaluation

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INTRODUCTION

Powdery mildew is a disease caused by a fungal, *Erysiphe necator*, which is an obligate parasite of most grapevine (*Vitis vinifera* L.) varieties [1]. The grape powdery mildew was first reported in North America in 1834 [2], China in the 1950's and in Africa in 1880 [3]. Since then the disease has become the most important disease of grapevine [3]. The disease can reduce 50-70% of yield and 20% wine quality by causing off flavours [4, 5]. The disease infect green leave tissues of grapevines, leaves appear white or greyish to white powdery, covering upper and lower surface of the leaves [3]. Under severe infection, leaves dry out and drop prematurely, plant growth is stunted and affected shoots appear dark brown to black with association of lesions [6]. Grapevine fruits of the infected plant are covered with white powdery, sometimes dark or dusty powdery, resulting in shrivelling of the fruit [7, 8]. This affects the quality appearance of the fruits, and eventually when such fruits are processed, it induces off flavours to wine. The pathogen *E. necator* survives as dormant haustoria in buds or as ascospores inside the thick-walled overwintering structures called chasmothecia. The infection starts when mycelium emerges from buds during initial growing, and then infects leaves [9]. The development of powdery mildew is seasonal and favoured in temperature ranging from 18-30°C [5]. The cleistothecia develop and become mature in the late summer and fall anywhere on the vine that mildew takes place [3]. They are washed from the vine and spurs with fall and winter rainfall and remain in soil until spring when they open and release their ascospores in response to rainy season [9]. Ascospores and/or mycelium growing out of infected buds are the primary sources of inoculum for new infection each year [10]. Under favourable conditions, production of conidia can occur within 7-10 days after primary infection. This cycle continues throughout the seasons as long as moderate temperature between 18-30°C persists [5]. For decades, fungicides such as sulphur have been used to control powdery mildew in grape [3]. However, its effectiveness depends on the weather condition; under heavy rainfall, application of sulphur is washed down and therefore reduces its effectiveness [11]. The effectiveness of sulphur as a common fungicide is limited at the temperature below 15°C and above 35°C [12] and some of fungal strains of *E. necator* have been reported to develop resistance [13, 14]. Because of this, the severity of the powdery mildew to grapevine has been increasing due to development of resistance to *E. necator*. In addition, continuous use of fungicides is not only costly but affects the environment. Therefore, development of grapevine varieties which are resistant to powdery mildew is the only cost-effective and environmentally friendly approach towards management of the disease [1]. This study therefore, aims to screen grape varieties for resistance to powdery mildew in order to introduce appropriate traits which may be useful for the development of grapevine resistant varieties.

MATERIALS AND METHODS

Location of the study

The study was conducted at Makutupora Agricultural Research Institute (Longitude: 35°, 46.093'E and Latitude: 05°, 58.669' S) (Altitude: 1080m) in Dodoma, Tanzania from 2014 to June, 2015.

Plant materials

Ten grapevine varieties; Queen of vine yards, Halili Belyji, Regina, Black rose, Makutupora red, Syrah, Chancellor, Ruby seedless, Alphonse Lavallee, and Makutupora white including control were selected for screening against resistance to powdery mildew. In order to minimize contamination, soils were sterilized then potted in polythene bags. Grapevine local variety Makutupora white was used as a control in our experiment because; it has been reported to be tolerant to powdery mildew (In communication with Research Director- Makutupora Agricultural Research Institute, 2014). Seedlings at three months raised in the potted polythene bags were then ready to be inoculated.

Experimental design

Complete Randomized Block Design (CRBD) with 3 replications for each variety was used to assess the resistance of 10 grapevine varieties including the control to powdery mildew. CRBD was used due to its precision, no restriction on the number of treatments or replicates and missing plots can easily be estimated. CRBD was adopted in this experiment to observe differences between treatments in this case inoculation procedures against resistance of grapevine varieties. Each replication had two blocks and four cuttings per variety were planted in each block. Each cutting was planted in one plastic pot containing sterile soil. Plants at three months of age in the first block were treated with dry inoculum and the second block was treated with suspension inoculum of *E. necator*. Routine management involving regular irrigation and other management activities such as fertilizer application were effected.

Source of inoculum

Dry *E. necator* inoculum of powdery mildew was prepared from diseased leaves of Alphoncelavalle a susceptible variety of grapevine to powdery mildew collected from Agricultural Research Institute, Makutupora. Leaves were grounded and packed as described by [15]. Spore density per gram of inoculum was calculated based on [16] using haemocytometer. Inoculum was inoculated to healthy plants of each first block per replication by gently shaking perforated tin containing measured amount of dry-inoculum on top of the leaves during the evening hours. Approximately 2×10^5 spores per ml were applied per plant. Prior to inoculation, plants were sprayed with water because fungi spores germinate readily under a film of moisture. The powder prepared after grinding were also used to prepare the suspension. The recommended 2×10^5 spore/ml based on [17] was calculated for spraying on healthy plants on each second block per replication. The suspension of inoculums was prepared at Tanzania Coffee Research Institute (TaCRI) laboratory in Kilimanjaro region and was transported to Dodoma for inoculation using ice box.



Evaluation of disease severity

The disease severity was determined based on [18, 19] using disease scale of 0-5 where 0 - Immune = 0%, 1- Highly resistant = 0.1 to 5%, 2 - Resistant = 5.1 to 10%, 3 - Tolerant = 10.1 to 25.0%, 4 - Susceptible = 25.1 to 50%, 5 - Highly susceptible = 50.1 to 100%. Data was recorded based on the appearance of powdery mildew and white spots on leaves.

Statistical method of data analysis

The data was analysed using GENSTART-software 15th edition, the general analysis of various (ANOVA) was used to test the severity effect on grapevine and the inference was made based at significance level of 5%.

RESULTS AND DISCUSSION

Disease incidence of grape varieties inoculated with suspension and dry *Erysiphe necator* isolates

Grapevine plants treated with dry leaf and suspension inoculation showed disease symptoms on leaves during the second week after inoculation. Infection slightly increased with number of days and it reached at peak during fifth week after inoculation. Results showed significance difference ($P < 0.05$) in the level of infection (Table 1 and 2).

Table 1. Rate of severity of grapevines to powdery mildew as per inoculation method.

Key: AL- Alphonse lavallee, CH- Chancellor, D- Dry, HA- Halili belyji, MR- Makutupora red, MW- Makutupora white, QN- Queen of vineyards, RG- Regina, R- Resistant, RU- Ruby seedless, SE-Sensitivity, S- Susceptible, SU- Suspension, SY- Syrah, T- Tolerant, RT- Rating, VR-Variety

| VR | SU inoculation method | | | D inoculation method | | | Severity of SU and D inoculation method | | | | |
|----|-----------------------|----|----------------------|----------------------|----|----|---|----|----|----|--------------------|
| | SE | RT | Mean | VR | SE | RT | Mean | VR | SE | RT | Mean |
| CH | 2 | R | 2.33 ^a | CH | 3 | T | 3 ^a | CH | 2 | R | 2.67 ^a |
| SY | 3 | T | 2.67 ^{ab} | RG | 3 | T | 3 ^a | SY | 3 | T | 3 ^{ab} |
| MR | 3 | T | 3 ^{abc} | QN | 3 | T | 3 ^a | RG | 3 | T | 3.17 ^{ab} |
| MW | 3 | T | 3 ^{abc} | MR | 3 | T | 3.33 ^a | MR | 3 | T | 3.17 ^{ab} |
| RU | 3 | T | 3 ^{abc} | MW | 3 | T | 3.33 ^a | MW | 3 | T | 3.17 ^{ab} |
| HA | 3 | T | 3.33 ^{abcd} | SY | 3 | T | 3.33 ^a | HA | 4 | S | 3.5 ^b |
| RG | 3 | T | 3.33 ^{abcd} | AL | 4 | S | 3.67 ^a | RU | 4 | S | 3.5 ^b |
| BR | 4 | S | 3.67 ^{bcd} | HA | 4 | S | 3.67 ^a | QN | 4 | S | 3.67 ^b |
| AL | 4 | S | 4 ^{cd} | BR | 4 | S | 4 ^a | AL | 4 | S | 3.83 ^b |
| QN | 4 | S | 4.33 ^d | RU | 4 | S | 4 ^a | BR | 4 | S | 3.83 ^b |

Table 2. Analysis of variance of severity among inoculation types. Key: d.f- degree of freedom, e.s.e- Standard errors of means,Fpr- statistical probability,l.s.d- Least significant differences of means, s.e.s- Standard errors of differences means, m.s- mean square, s.s- sum of squares, v.r- variance

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------------|------|---------|--------|------|-------|
| Inoculation type stratum | 1 | 0.3788 | 0.3788 | 0.98 | |
| Inoculation type.*Units* stratum | | | | | |
| Variety | 10 | 7.8182 | 0.7818 | 2.03 | 0.048 |
| Residual | 54 | 20.7879 | 0.3850 | | |
| Total | 65 | 28.9848 | | | |

e.s.e= 0.2533, s.e.d= 0.3582, l.s.d at (5% level)= 0.7182,



The methods of estimation of variety resistance in our study using established seedlings from cuttings in the field conditions have previously been used by [20]. The grape variety, Alphonse lavallee, showed the highest level of disease incidence (33.30%) and Chancellor indicated the lowest (11.15%) (Table 3). The present study indicated significance difference ($P < 0.05$) of disease incidence among grapevine varieties tested (Table 4).

Table 3: Disease incidence on grapevine as per inoculation method. Key: AL- Alphonse lavallee, CH- Chancellor, D- Dry, HA- Halili belyji, MR- Makutupora red, MW- Makutupora white, QN- Queen of vineyards, RG- Regina, RU- Ruby seedless, S-Suspension, SY- Syrah

| Incidence | | Incidence | | Average | |
|------------------------|---------------|-----------------|---------------|---------|---------------|
| Suspension Inoculation | | Dry inoculation | | | |
| Variety | Incidence (%) | Variety | Incidence (%) | Variety | Incidence (%) |
| CH | 8.98 | CH | 13.33 | CH | 11.15 |
| MW | 13.79 | MR | 16.10 | MW | 15.39 |
| SY | 15.53 | MW | 16.99 | SY | 16.51 |
| RG | 18.86 | QN | 19.36 | MR | 19.65 |
| MR | 21.01 | RG | 22.46 | RG | 20.66 |
| RU | 21.37 | SY | 22.62 | RU | 27.70 |
| HA | 24.93 | AL | 27.32 | BR | 27.84 |
| BR | 26.64 | BR | 29.03 | HA | 28.65 |
| AL | 39.27 | HA | 32.37 | QN | 32.35 |
| QN | 45.34 | RU | 34.02 | AL | 33.30 |
| Mean | 21.42 | | 21.23 | | |

Table 4. Analysis of variance of incidence among inoculation types. Key: d.f- degree of freedom, e.s.e- Standard errors of means, Fpr- statistical probability, l.s.d- Least significant differences of means, s.e.s- Standard errors of differences means, m.s- mean square, s.s- sum of squares, v.r- variance.

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|----------------------------------|------|---------|---------|------|-------|
| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
| Inoculation type stratum | 1 | 0.00001 | 0.00001 | 0.00 | |
| Inoculation type.*Units* stratum | | | | | |
| Variety | 10 | 0.32602 | 0.03260 | 2.21 | 0.031 |
| Residual | 54 | 0.79835 | 0.01478 | | |
| Total | 65 | 1.12438 | | | |

e.s.e= 0.0496, s.e.d= 0.0702, l.s.d at (5% level)= 0.1407

These results demonstrate this difference in susceptibility to the powdery mildew disease could be due to the difference in the genetic constitution of grapevine varieties evaluated. Grapevine variety Chancellor can be used as pure line to produce vines or in cross breeding to produce hybrid variety. Similar variety was published previously by [21] to be resistant to powdery mildew however; most of our varieties were susceptible to disease. However, [22] reported that, grape varieties with resistance to powdery mildew are currently being developed, using either conventional or transgenic approaches, each of which has associated advantages and disadvantages. Previous studies by [12, 5, 18, 19,] who carried out the same studies on evaluation of grapevines in resistance to powdery mildew disease identified some breeding varieties which are immune, highly resistant, resistant, susceptible and highly susceptible. Similarly, [19, 23, 24, 25, 26, 5] reported variations in terms of the level of resistance of grapevines. The varieties Black rose and Alphonse lavallee which demonstrated high susceptibility to disease have been reported to be of high yielding and excellent flavour quality [27]. These can be crossed to varieties such as Chancellor to produce hybrids which will have combined traits of disease resistance, high yielding and excellent flavour quality. This is in agreement with previous studies which reported that susceptible varieties of *Vitis vinifera* cultivars can be introgressed to varieties with resistant traits to produce hybrid varieties which on top of excellent attributes they have hybrid [25, 20, 6, 3, 1]. Producing new varieties with several disease resistance including powdery mildew helps to keep plant health and maintain its sugar content in fruits and avoids damages from other pests thus maintain original quality [5, 28].



Effectiveness of suspension and dry inoculation methods in screening for resistance to powdery mildew

The results of the present study showed that suspension inoculum method creates higher selection pressure than dry leaf inoculation method. The possible reason could be due to availability of optimum condition for the pathogen to grow, the previous study reported that, the spores of *E. necator* grow well in presence of moist condition and temperature ranging from 18°C to 30°C which favour sporulation [29, 30]. These results are complementary with other authors who have been documented that, when using these two methods of screening grapevine samples against resistance to powdery mildew, suspension *E. necator* inoculum has shown high disease severity than dry inoculation method, [18, 19] reported the similar results by comparing the differences in the effectiveness of these two inoculation methods of which suspension method responded positively. However, [1] on his study, dry inoculation showed more efficient compared to wet and drop inoculation methods used. The infection efficiency may be important component of rate reducing resistance; suspension inoculation method has been reported to be more efficiency on sporulation of inoculum where, the earlier of the first symptoms of the pathogen appeared, the more severe powdery mildew infection was observed on the leaves and clusters [3].

CONCLUSION AND RECOMMENDATION

This study demonstrated significant variation among different varieties of grapevines in resistance against powdery mildew. The resistant varieties can be used as pure lines or to introgress with local susceptible grapevines which have attributes of high yielding and excellent flavour quality to produce hybrid varieties, to achieve this, new varieties developed must be well managed such that leaves falling, uneven maturation, swelling and cracking which finally lead to fruit rot, yield depression and decline in wine quality in terms of sugar content should be avoided. Among of the methods used for inoculation in our study, we recommend suspension as the best method for inoculation because the penetration of inoculum through leaves is high thus giving relevant outcomes. Among the varieties screened, Chancellor showed high level of resistance to powdery mildew. This variety can be used as pure commercial cultivar or to cross breed in order to produce hybrid variety using either conventional or transgenic approaches. The susceptibility to powdery mildew generally was high to local varieties compared to improved varieties therefore; local varieties should be kept in the gene bank as the resource for future use. Most of improved varieties were resistant to disease. However, we recommend further studies of screening powdery mildew for horizontal resistance in order to introduce grape varieties with resistance to several diseases. We recommend that in addition to Chancellor, wild species should be used as the valuable source of resistance to powdery mildew meanwhile new improved varieties should be in process to contribute high production. In addition; the disease evaluation should be confirmed in the laboratory under controlled condition.

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