Northern Territory Government
Department of Business, Industry & Resource Development

# Characterisation of the Grapevine Leaf Rust Fungus and Identification of Resistant Grape Cultivars (Project 1A)


## FINAL REPORT TO:

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **NT02-01**

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Research Organisation: **DBIRD**

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## TABLE OF CONTENTS

## *EXECUTIVE SUMMARY*

Some preliminary investigations were conducted in Project 1A to enable ensuing research on the Grapevine Leaf Rust pathogen, *Phakopsora euvitis*. Objectives of this project included identifying a selection of grapevine types that could be procured for resistance/immunity screening and fungicides to be assessed for their ability to control the pathogen. The list of grapevines includes more than 430 cultivated (including rootstock and hybrid selections) and native types. The fungicides selected for assessment (12 in total) include some already registered for use on grapevines and others shown to be effective against rust diseases on other crops. They have a number of modes of action, encompassing both systemic and protective systems. The ability to provide replacement resistant/immune vines and information about fungicides that effectively control *P. euvitis* will play a vital role in the future bio-security of viticulture related industries.

The methodology for a PCR-based DNA analysis of *P. euvitis*, developed by Ono and Imazu (2001), is outlined in this report. The ability to conduct a DNA analysis could play an important role in establishing the identity of possible re-incursions in Darwin and new incursions in other parts of Australia.

A further objective was to inoculate some potted grapevines with *P. euvitis* to provide a ready source of fresh inoculum for the continued resistance/immunity screening of grapevine cultivars. The attempts at inoculation during this project were unsuccessful so data from a previous successful attempt has been included in this report. The inoculated grapevines were rated for both disease severity and incidence. All were given an average disease severity rating of either 1 or 2 indicating that on average, either less than 1% or 1 – 5% of the total leaf area on each grapevine was affected by symptoms of *P. euvitis*. Statistical analyses of this data indicated a significant difference in disease severity existed generally between cultivars. The variation in severity of infection from leaf to leaf on each variety was generally small. The incidence of disease on each of the inoculated cultivars ranged from 34 percent of leaves infected on "Red Emperor" to 90 percent of leaves infected on "Ruby Seedless". This provides important information about the susceptibility of *Vitis vinifera* cultivars, which almost all viticulture related industry in Australia is reliant upon.

The final objective was to determine a suitable method of leaf disc culture that could be employed for conducting research with *P. euvitis*. Various methods used in previous studies were assessed to determine which achieved the most desirable results ie, practicality and longevity of leaf survival. The assessments showed that leaf discs mounted on cotton wool soaked with sterile distilled water and enclosed in petri dishes was practical and consistently lasted for longer periods of time than any other method.

## *Background*

Grapevine leaf rust (GLR) was discovered in Darwin in July 2001 (Weinert *et al.* 2003). The disease, caused by the fungus *Phakopsora euvitis*, did not occur previously in Australia. Surveys in Katherine and Ti Tree, approximately 200 kilometres north of Alice Springs, and all other Australian states have determined these areas to be GLR free. There are no commercial vineyards in the Darwin region. Grapevines are grown by householders, principally for their leaves to make Dolmades.

GLR disease is common in Asia and Central America and can be very destructive if not controlled (Leu 1988). It is windborne, and has a high potential for spread by humans and machinery. It can infect grapes over a range of temperatures and there is a reasonable chance that it could establish in the majority of Australia's viticulture regions.

## *Objectives*

1. Identify and draft a methodology for the PCR-based characterisation of *P. euvitis*.
2. Infection of potted grapevines with *P. euvitis* for an inoculum source and disease rating.
3. Identify fungicides with known or potential activity against rust diseases.
4. Identify sources of grapevine germplasm that will later be tested for resistance/immunity to *P. euvitis*.
5. Develop and test methods based on leaf disc culture to later be used for mass screening of grapevine germplasm for resistance/immunity to *P. euvitis* and fungicide efficacy assessment.

## *Methods*

*Identification and drafting of a methodology for the PCR-based characterisation of P. euvitis.*

- The methodology outlined in this report is that of Ono and Imazu (2001) (see results).

*Infection of potted grapevines with P. euvitis for an inoculum source and disease rating*

- Spores of *P. euvitis* were suspended in a mixture of sterile distilled water (SDW) and 2 drops/mL of Tween 80®.
- Two plants of each cultivar had of their leaves sprayed, predominantly on the adaxial side, with the spore suspension (concentration of approximately 30 to 40,000 spores/mL) atomised using a Preval® spray pack. One control plant per variety was sprayed with SDW mixed with 2 drops/mL of Tween 80®.

- The plants were covered in plastic bags for 24 hours to assist infection.
- Infection with *P. euvitis* was evident seven days after inoculation. 14 days after inoculation the infection was well advanced and rated for incidence and severity.
- The disease incidence was rated based on the percentage of leaves infected per vine, and the disease severity rated based on the average leaf area (LA) covered by pustules and associated chlorotic/necrotic symptoms of *P. euvitis*. This was rated on a scale from 0 to 5 where 0 = no infection, 1 = <1%, 2 = 1-5%, 3 = 6-10%, 4 = 11-15% and 5 = >15% of LA covered.
- The disease severity data (excluding that for the "Perlette" cultivar) was analysed by comparing the medians (Median Test) and by comparing ranks using a Kruskal-Wallis non-parametric ANOVA. The disease incidence data was analysed using a generalised linear model (for binomial errors with a logit link).

*Identification of fungicides with known or potential activity against rust diseases.*

- Fungicide selection was based on examination of available literature on other rust species and the Infopest AGVET and Australian Pesticides and Veterinary Medicines Authority registered products databases.

*Identification of sources of grapevine germplasm that will later be tested for resistance/immunity to P. euvitis.*

- Identification and selection of grapevine selections/cultivars was based on reported resistance to *P. euvitis* and the availability of material.

*Development and testing of methods based on leaf disc culture to later be used for mass screening of grapevine germplasm for resistance/immunity to P. euvitis and fungicide efficacy assessment.*

- Four basic methods including ones used in previous studies by Evans *et al.* (1996), Johnston and Scott (1988) and Washington (1987) were tested and compared. They were:-
  1. leaf disc on cotton wool soaked in sterile distilled water, sealed in a petri dish,
  2. leaf disc on water agar, sealed in a petri dish,
  3. leaf disc floating in sterile distilled water, sealed in a petri dish and,
  4. detached leaf with only the petiole in water, sealed in a plastic bag.
- The methods were assessed using four leaves for each, checked daily for senescence. Leaves were considered non-functional after 50% of the leaf area had become chlorotic.

## *RESULTS/DISCUSSION*

*PCR methodology (Ono and Imazu 2001).*

DNA Extraction

- Vacuum 1-3mg urediniospores from leaves.
- Crush spores and suspend in 30μL of extraction buffer.
- Incubate for 3 hours at 55°C, before heating for 10 minutes at 95°C.
- Dilute with 30μL of distilled water.

Amplification

- The reaction mixture contains 5μL DNA suspension, 200 μM of dATP, dCTP, dGTP, and dTTP, 1mM of $MgCl_2$, 50mM KCl, 0.2μM of primer, 1.25 units of AmpliTaq Gold® DNA polymerase.
- Complete 45 cycles consisting of an initial 12 minutes at 95°C, followed by 1 minute cycles at 60°C, 72°C and then 30 seconds at 95°C and ending with an extension at 72°C for 10 minutes.
- The DNA is then digested for three hours and electrophoresed on an 8% polyacrylamide gel in TBE buffer.

The ability to conduct a DNA analysis could play an important role in establishing the identity of possible re-incursions in Darwin and new incursions in other parts of Australia. It is hoped this will also assist in differentiating local strains from each other (should more than one exist) and from those in close proximity eg, East Timor.

*Disease Ratings*

During this project a viable source of *P. euvitis* inoculum was not available and attempts to inoculate the potted grapevines were unsuccessful. Therefore, it was decided to include data recorded following a previous successful inoculation in this report. The successful inoculation included 14 table grape varieties that were subsequently rated for disease incidence and severity (Table 1). This provides important information about the susceptibility of *Vitis vinifera* cultivars, which almost all viticulture related industry in Australia is reliant upon. These cultivars all showed symptoms of infection (chlorotic spots and pustules) seven days after inoculation. 14 days after inoculation the disease was well advanced in all cultivars when the disease ratings were conducted.

Table 1 indicates that differences in the severity of disease between cultivars were small. However, statistical analyses of this data showed that generally a significant difference in disease severity existed between cultivars. Pair-wise comparisons for the cultivar ranks (K-W ANOVA) indicated that "Red Emperor" and "White Muscat" were different from all other cultivars with respect to the disease severity. "Thompson's Seedless" was only different from these two cultivars. Each remaining cultivar was generally different from half or more of the other cultivars in the disease severity recorded. At a glance the data in Table 1 seems to indicate differences in disease incidence between cultivars. However, this data was analysed using a generalised linear model (for binomial errors with a logit link) which showed there was no significant difference ($p=0.08171$). A larger sample size would have been more informative and would probably have lead to statistical differences between cultivars. Potential differences in disease incidence and actual differences in disease severity are probably a reflection of a variable infection rate due to the inoculation process (discovered since that grapevine leaves are penetrated by *P. euvitis* infection pegs via the abaxial surface only) rather than a difference in susceptibility of the cultivar. Differences in the proportions of leaves of a particular age on the vines may also contribute as they vary with age in their susceptibility to *P. euvitis* (the very young, soft leaves seem to be the least susceptible). None of the control plants showed symptoms of infection with the pathogen.

**Table 1. Incidence and severity of GLR disease on varieties of table grape**

| Cultivar | Disease Incidence (%) | Highest Disease Severity Rating | Average Disease Severity Rating |
|---|---|---|---|
| Perlette | 67 | 3 | 2 |
| Thomuscat-1 | 82 | 4 | 1 |
| Thomuscat-2 | 73 | 3 | 2 |
| Pearl of Csaba-1 | 60 | 4 | 1 |
| Pearl of Csaba-2 | 76 | 5 | 2 |
| Red Prince-1 | 56 | 3 | 2 |
| Red Prince-2 | 61 | 4 | 2 |
| Ruby Seedless-1 | 76 | 3 | 2 |
| Ruby Seedless-2 | 90 | 4 | 2 |
| Black Muscat-1 | 64 | 4 | 2 |
| Black Muscat-2 | 77 | 2 | 1 |
| Thompson's Seedless-1 | 72 | 3 | 2 |
| Thompson's Seedless-2 | 78 | 3 | 1 |
| Red Emperor-1 | 58 | 2 | 1 |
| Red Emperor-2 | 34 | 2 | 2 |
| White Muscat-1 | 41 | 3 | 1 |
| White Muscat-2 | 47 | 2 | 1 |
| Black Sultana-1 | 55 | 2 | 1 |
| Black Sultana-2 | 71 | 4 | 1 |
| Emerald Seedless-1 | 72 | 3 | 1 |
| Emerald Seedless-2 | 51 | 3 | 1 |
| Flame Tokay-1 | 71 | 3 | 2 |
| Flame Tokay-2 | 45 | 4 | 2 |
| Sultana M12-1 | 63 | 3 | 2 |
| Sultana M12-2 | 63 | 3 | 2 |
| Ladies finger-1 | 64 | 4 | 2 |
| Ladies Finger-2 | 65 | 4 | 2 |

NB. 30% (approx.) was the highest recorded symptom coverage on any one leaf.

*Fungicides With Known or Potential Activity Against Rust Diseases*

Below (Table 2) are fungicides identified as potential chemical control agents for *P. euvitis.* They have a variety of modes of action. It is important to utilise this diversity during spray programs to avoid a build-up of resistance to the fungicides. Assessments of these fungicides will provide important information about their efficacy should chemical control be required as part of a response to any future outbreaks.

**Table 2. Fungicides to be assessed for their efficacy against *P. euvitis***

| Trade Name | Active | Mode of Action | Group | Comments |
|---|---|---|---|---|
| Impact | Flutriafol | Systemic | A | Used on blackberry rust |
| Dithane | Mancozeb | Protectant | Y | Used on blackberry and wheat rust |
| Tilt | Propiconizole | Systemic | C | Used on blackberry and wheat rust |
| Bayleton | Triadimefon | Systemic | C | Used on wheat rust |
| Plantvax | Oxycarboxin | Systemic | G | Used on rust diseases |
| Amistar | Azoxystrobin | Protectant / Systemic | K | Registered for use on grapevines |
| Wettable Sulfur | Sulfur | Protectant | Y | Registered for use on grapevines |
| Champ Dry Prill | Cupric Hydroxide | Protectant | Y | Registered for use on grapevines |
| Marvel | Benomyl | Systemic | A | Used in leaf disc trials. Inhibited germination of *P. euvitis* |
| Bravo | Chlorothalonil | Protectant | Y | Found to be the most effective against *Puccinia psidii.* |
| Mycloss | Myclobutanil | Protectant / Systemic | C | Used on grapevines. Active against many rust fungi. |

*Grapevine Germplasm*

The following is a list of names of the cultivars to be screened for resistance/immunity to *P. euvitis.* The ability to provide replacement resistant/immune vines will play a vital role in the future bio-security of viticulture related industries. These cultivars were obtained from SARDI - Nuriootpa, CSIRO - Merbein and Viticlone Supplies Nursery – Margaret River. They include wine grape, table grape and rootstock types. At the bottom of the list are native genera of *Vitaceae* occurring in the Top End. The majority of the cultivars are *Vitis vinifera*, which make up the bulk of grapevines grown in Australia. The selection however incorporates as many rootstock and hybrids as possible, as these plants tend to have qualities such as greater disease resistance, general hardiness and vigour. Finally there are a number of cultivars listed that have been assessed for susceptibility to *P. euvitis* in previous studies. Unfortunately, these studies are somewhat

contradictory which may be a result of inaccurate identifications or assumptions of the specie(s) of rust causing the disease.

Variety/Clone:-

106.8
1202
1613
1616
3306
99 Richter
101-14 - HT 100-3
107-11
10868 Seibel
128 Seibel
188-04 Castel
21 B Trier
333 EM Foex
34 EM
34 EM
41 B
420 A
554-5 seedlings
62-66
A x R 1
Abouriou
Agadaj
Agawam
Agestsage Blanc
Alden (R)
Aleatico
Aledo
Alicante Bouschet
Americano (?)
Angostenga Blanc
Ansonica
Antigona
Aramon
Arinarnoa
Arneis 15 CVT
Arrilobe
Aualdena No. 1
Auldana No. 3
Aurelia
Aurore
Autumn Black
Auxerrois
Baco Blanc - C10V12
Baco Noir
Baileys Aucerot #1
Balluti (B)
Banatski Muskat
Bankside Acorn
Barbera
Baresana
Barlinka
Baroque
Bastardo
Baufranc
Baxter's Sherry
Beauty Seedless
Bedgradske Besemena
Bellino X
Bianca D'Allessane
Biancolella
Biancone
Bicane
Black Alicante
Black Frontignac - BVRC12
Black Malaga
Black Mammoth
Black Muscat
Black Sultana
Blush Seedless (88-03)
Boal
Bonvedro Cl.146
Brown Frontignac - LC 2
Buckland's Sweetwater
Burgrave X
C.G. 26-879
Cabernet Franc
Cabernet Franc Francese
Cabernet Sauvignon
Calitor Noir
Calmeria
Campbell's Early
Canada Muscat
Canadice
Canner
Canocazo
Canon Hall Muscat
Cape Currant
Cardinal
Carignan
Carina
Carmine
Carnelian
Carolina Blackrose

Cascade
Catawba (SC-OR-SC)
Cayuga White
Golden Muscat
Centennial Seedless
Centurion
Cesanese
CG 1481 (W)
CG 1730
CG 4320 (R)
Chambourcin
Chancellor (S.7053)
Chardonnay
Chasan INRA
Chasselas Dore
Chenin Blanc
Christmas Rose C
Cinsaut
Clairette
Clairette Blanche (F)
Clersole Logine
Colombard
Concord (SC-OR-SC)
Constantia (F)
Corvina Veronese
Couderc Noir
Crimson Seedless
Criolla negra
Crouchen
Crystal
Daira Seedling
Danlas (W)
Danugue
Dawn Seedless (88-05)
De Chaunac
Delaware
Delight

Demir Kapija
Diamond
Dizmar (W)
Djandal Kara (B)
Dog Ridge
Dolcetto
Doradillo
Dourado
Durif
Dutchess
Early Muscat
Egiodola
Elvira
Emerald Riesling
Emerald Seedless
Emperor - B9V5
Enhresfelser
Exotic
Fantasy Seedless
FER
Fercal
Fernao Pires
Fetyeska
Fiano
Fiesta
Flame Seedless
Flame Tokay
Flora
Foch
Folle Blanche BGW No. 16
Freedom
Freisa
Fresno 27-31 (B)
Fuji Muscat (W)
Furmint
Gamay – Beaujolais 200A
Ganson

Garronet
Gascon
Glenora
Gold
Gouais
Goyura
Graciano
Gramon
Granache BVRC 5
Grec Rose
Green Veltliner
Greg Rose
Grenache
Grocanica
Gropello Gentile
Gros Colman
Gros Meslier
Harmony
Harslevelu
Helena
Henab Turki
Heptakilo
Herbemont
Herbert
Himrod
Hunisa (B)
Illinois 547-3 A133
Iona local (grafted on Dogridge)
Irsay Oliver (W)
Isabella
Italia
J 17-48
J S 23-416
J17-69
Jacquez
K 51-40
K51-32

Kadarka
Kavadarski Drenak
Keknyelu
Parsley Leaf Chasselas
Kishmishi
Kober 125 AA
Kober 5BB
Kyoho
Lady Downe's Seed
Lady Patricia
Lady's Finger
Lagrain
Lambrusco H9V12
Leon Millot
Les de L'el
Lider 171-13
Lignan
Lilierila INRA
Limberger
LN 33 – Student
Loose Perlette (W)
Maccabeu
Madresfield Court
Malbec
Malta Seedless (8275)
Malvasia Bianca
Malvasia Istria
Mammolo
Mantley 8123
Marechal Foch
Marroo Seedless (B)
Marsanne
Mataro
Melon
Melvasia Rei
Menavacca (B)
Merbein Seedless
Merlot
Meunier
MH 29-56
Mission Seedling
Molinara
Mondeuse
Monerac
Montepulciano
Montils
Monukka
Morio Muscat
Moss Sultana
Mrs. Pince's Muscat
Mtsvase
Mueller Thurgau
Muscadelle
Muscadelle du Bordelaise (F)
Muscat a petits grains
Muscat Blanc – F3V14
Muscat Gigas
Muscat Gordo Blanco
Muscat Hamburg
Muscat Ottenel
Muscat Rouge
Nebbiolo 111 CVT
Nebbiolo K6V1
Nebbiolo Bourgu
Nebbiolo Fino
New York Muscat (R)
Nyora
Odola
O'Hanez
Olivette Noir
Ondenc
Opuzensia Rana
Orange Muscat
Ortruge
P 76 - 19 (E4V8)
Palomino
Pannaonia Gold
Parellada
Paulsen 1045 – 01R
Paulsen 1103
Paulsen 779 – 01R
Pearl of Csaba
Pedro Ximenez
Perdea
Perle De Csaba
Perlette
Petit Meslier
Petit Verdot
Picolit
Pink Sultana
Pinot Blanc
Pinot gris
Pinot Noir
Piquepoul Noir
Portan
Procupak
Putzscheere
Queen
Quick's Seedling
R 99 (2-10-285)
Rabener
Raboso Piave
Radmilovaski Muscat
Raffiet de Moncade
Ramsey
Red Emperor
Red Globe (88-02)
Red Lady's Finger
Red Malaga
Red Palamino
Red Prince
Reichensteiner
Rhine Riesling
Ribier

Ribol (B)
Richter 110
Riesling 237 Gm
Riparia Gloire
Rkaziteli
Rolle
Rosaki
Rose Cross ex Drumborg
Rosulus
Rousanne
Royal Ascot
Royalty
Rubired
Ruby Cabernet
Ruby Seedless
Ruggeri 140
Rupestris St. George
Russian Seedless (B)
Sabalkenskoi (Red Ohanez)
Sangiovese
Santa Paula
Saperavi
Saturn Ex Northfield
Sauvignon Blanc
Sauvignonasse
Scarlet
Scheurebe
Schuyler (B)
Schwarzmann
Semebat
Semillon
Senecca
Seyval
Shiraz
Shtur Angur - I3V9
Siegarrebe
Smederevka
SO 4
SORI - 92-14
Souzao
Souzao
St. Macaire
Suffolk Red
Sugraone
Sultana (H12)
Sultana Denham Sport
Sultana H 25
Sultana M12
Sultana Moschata
Sultanina Monococco
Sumoll
Sylvaner
Symphony
Taminga
Tandannya
Tannat
Tarrango
Teleki 5A
Teleki 5C
Teleki 8B
Tempranillo
Temprase
Teroldego
Terret Noir
Thompson's Seedless
Thomuscat
Tinta Ameralla
Tinta Cao
Tinta Carvalha
Tinta Molle (Madeira)
Touriga
Touriga ex Rutherglen
Trabbiano LRC 15
Trajadura
Traminer
Traminer X Riesling
Trebbiano
Trentham Black
Trieste 4X
Trollinger
Tulillah
Tunn Currant
Ughetta
Urbana
Valdepenas Tempranillo
Valdiguie
Valensi Blanc
Varousset
Venus - B
Verdelho
Verdelot
Verdicchio
Villard Blanc
Villard Noir - Q106-5Sb
Viognier
*Vitis amurensis*
*Vitis berlandieri*
*Vitis candicans*
*Vitis caribaea*
*Vitis cordifolia*
*Vitis labrusca*
*Vitis longii*
*Vitis riparia*
*Vitis rotundifolia*
*Vitis rotundifolia*
Waltham Cross
White Muscat
Wood's Red Muscat
Xarelle
Zante Corinth
Zante Currant - BC 0158
Zinfandel

The native *Vitaceous* genera found in the Northern Territory are *Cissus adnata, Cissus rotundifolia, Ampelocissus acetosa, Ampelocissus frutescens, Cayratia acris, Cayratia maritima, and Cayratia trifolia.* However, not all of the native vines are common in the Darwin region so their availability will determine which ones are tested. *P. euvitis* is specific to plants in the genus *Vitis* that does not include native grapevines. Therefore, it is not expected to be able to establish in these populations but it will be important to assess them thoroughly to ensure they are not potential hosts.

*Leaf Disc Culture*

The objective was to assess methods that would provide a practical means of mass screening grapevine cultivars for resistance/immunity, assessing fungicides for their efficacy and also provide adequate longevity of leaf survival. Leaf discs mounted on cotton wool was chosen as the most consistent method and leaves remained viable for a longer period of time than with other methods. Leaf discs mounted on water agar (WA) was the next best method, as shown in Table 3 below:

**Table 3. Assessment of methods of detached leaf culture**

| Detached leaf method | Av. Viable days | Comments |
|---|---|---|
| Whole leaf with petiole only in water | 14 | Very inconsistent. Petiole tends to become weak and drop off, killing the leaf. |
| Leaf disc in petri dish on cotton wool | 23 | Consistent. All leaves lasted to 23 days, 3 to 27 |
| Leaf disc in SD water in petri dish | 21 | Inconsistent. One developed roots. |
| Leaf disc on WA | 20 | Consistent. Leaves develop fungi and bacteria without the addition of lactic acid in the water agar. |

Some trials were conducted using WA amended with Benomyl as this has been reported to improve the survival of detached leaves. However, it was found that germination of *P. euvitis* spores was effectively eliminated when streaked onto plates of WA with the fungicide incorporated and as such could influence the results during screening of cultivars for resistance or immunity.

## *OUTCOME/CONCLUSION*

With the exception of infecting potted grapevines with *P. euvitis* to provide a ready source of inoculum for continued resistance/immunity screening of grapevine cultivars, the objectives of Project 1A in which preliminary investigations were carried out to enable ensuing research on GLR have been achieved. These included determining grapevine cultivars to be tested for resistance or immunity, fungicides to be assessed for their efficacy of control, identifying a methodology for DNA analysis, providing disease ratings of infected table grapes and identifying a suitable method of leaf disc culture. The unsuccessful attempt to produce a source of inoculum using the potted grapevines will not hamper the continued screening for resistance/immunity as the inoculum can be perpetuated *in vitro* using leaf discs. Due to the successful outcomes of these preliminary investigations, various aspects of research on GLR viewed as necessary for the future bio-security of viticulture related industries is able be conducted.

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***BUDGET RECONCILIATION***

| Budget | Funding required from GWRDC | | Actual Expenditure |
|---|---|---|---|
| Technical Assistant | 6,720.00 | | 6,720.00 |
| Operating | 900.00 | | 900 |
| | | | |
| | | | |
| | | | |
| | | | |
| Total | 7,620.00 | | 7,620.00 |
| Add 10 % GST | 762.00 | | 300 |
| **Total funds requested from GWRDC** | 8,382.00 | **Total Expended** | 8,382 |
| | | | |
| | | **Remitted to GWRDC** | 0 |
| | | | |

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