



Province Agriculture Laboratory  
Plant Protection and Pesticide Management Section  
Molecular Laboratory Section  
Gandaki Province, Pokhara  
**PCR Analysis Report FY 2082/083**

**Method:** The HLB diagnosis was performed by following the protocol prepared by Plant Protection Laboratory, Gandaki Province, Nepal based on DNA extraction using iRupt 24p tissue-lyser aided Zymo Research's Quick-DNA Fungal / Bacterial Miniprep Kit followed by quantification in BioTek Nanodrop, amplification in Bio-Rad thermal-cycler and gel visualization on 320 nm UV. Detection was based on Clas specific 500bp amplicon-based screening with primers:

Name of the Primer	Sequence (5'-3')
Forward (Las606)	GGAGAGGTGAGTGGAAATCCGA
Reverse (LSS)	ACCCAACATCTAGGTAAAAAC

**Tabulated Results:** (\*Date refers to the date when the sample was received by the laboratory)

**Sample ID Rule:** YY\_Nursary(N)/Orchard(O)\_Nursary.Sample.No/Sample.No

**Results:** +D (HLB detected: Samples showed presence of 500bp amplicon upon PCR), ND (HLB not detected upon PCR)

SN	Lab ID	Sample Identification Details	Date*	Location	Contact	Result
1.	82_O_01	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
2.	82_O_02	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
3.	82_O_03	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	ND
4.	82_O_04	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
5.	82_O_05	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
6.	82_O_06	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
7.	82_O_07	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
8.	82_O_08	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
9.	82_O_09	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
10.	82_O_10	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
11.	82_O_11	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
12.	82_O_12	Megh Bahadur BK	23/07/2082	Byas Mun 11, Tanahunshur	9815124216	+D
13.	82_O_13	Om Bahadur Jhadi Magar	24/07/2082	Newarbaari 06, Baudikali, Nawalpur	9844020670	ND
14.	82_O_14	Om Bahadur Jhadi Magar	24/07/2082	Newarbaari 06, Baudikali, Nawalpur	9844020670	+D
15.	82_O_15	Nirmala Sunaari	24/07/2082	Bobak 05, Baudikali, Nawalpur	9867476157	ND
16.	82_O_16	Harsa Bahadur Thapa, (2 <sup>nd</sup> Contact: Om Bahadur Chalmi Magar)	24/07/2082	Bobak 05, Baudikali, Nawalpur	9762241225	ND
17.	82_O_17	Suman Singh Aslami, (2 <sup>nd</sup> Contact: Om Bahadur Chalmi Magar)	24/07/2082	Bobak 05, Baudikali, Nawalpur	9762241225	ND
18.	82_O_18	Senadir Magar Rana (2 <sup>nd</sup> Contact: Nar Bahadur Rana)	24/07/2082	Karkot, Nawalpur	9843749964	ND
19.	82_O_19	Nar Bahadur Puara	24/07/2082	Karkot, Nawalpur	9843749964	ND
20.	82_O_23	Mani Saru Magar	24/07/2082	Bulingtar, Bhadauri	9766388283	+D

Analyzed by:

Mr. Sushil Dhungana  
Bio-technologist Officer

02-02-2082

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शालिकराम अधिकारी  
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(अधिकृतस्तर दर्शौ)



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21.	82_O_24	Durga Bahadur Saru Magar (2 <sup>nd</sup> Contact: Mani Saru Magar)	24/07/2082	Bulingtar, Bhadauri	9766388283	ND
22.	82_O_25	Mani Saru Magar	25/07/2082	Bulingtar, Bhadauri	9766388283	ND
23.	82_O_26	Durga Bahadur Saru Magar (2 <sup>nd</sup> Contact: Mani Saru Magar)	25/07/2082	Bulingtar, Bhadauri	9766388283	ND
24.	82_O_34	Ramesh Ale Magar (B1T1)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
25.	82_O_35	Ramesh Ale Magar (B1T2)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	ND
26.	82_O_36	Ramesh Ale Magar (B1T3)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
27.	82_O_37	Ramesh Ale Magar (B1T4)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	ND
28.	82_O_38	Ramesh Ale Magar (B2T1)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
29.	82_O_39	Ramesh Ale Magar (B2T2)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
30.	82_O_40	Ramesh Ale Magar (B2T3)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
31.	82_O_41	Ramesh Ale Magar (B2T4)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
32.	82_O_42	Ramesh Ale Magar (B3T1)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
33.	82_O_43	Ramesh Ale Magar (B3T2)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
34.	82_O_44	Ramesh Ale Magar (B3T3)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
35.	82_O_45	Ramesh Ale Magar (B3T4)	07/08/2082	Gunzara, Myagde RM 02, Tanahun	9818037529	+D
36.	82_O_46	Hum Bahadur Thapa Magar	07/08/2082	Benikot Vanjyang, Byas 13, Tanahun	9704089300	+D
37.	82_O_47	Manisha Thapa	07/08/2082	Benikot Vanjyang, Byas 13, Tanahun	9806667719	ND
38.	82_O_48	Deepak Thapa (2 <sup>nd</sup> Contact: Sukre Thapa)	07/08/2082	Benikot Vanjyang, Byas 13, Tanahun	9823083672	+D
39.	82_O_51	Upwards from Cold Room (Santosh Adhikari, AKC Baglung)	11/08/2082	Sigana 08, Baglung		ND
40.	82_O_52					ND
41.	82_O_53	Chandra Bahadur Thapa	11/08/2082	Thokanphat, Sigana	9766604610	ND
42.	82_O_54	Krishna Bahadur Pun	11/08/2082	Kathekhola 04, Baglung	9748407956	ND
43.	82_O_55	Krishna Bahadur Pun	11/08/2082	Kathekhola 04, Baglung	9748407956	ND
44.	82_O_56	Surya Prasad Neupane	11/08/2082	Kathekhola 06, Baglung	9847620428	ND
45.	82_O_57	Dhurba Kandel	11/08/2082	Kathekhola 06, Baglung	9846823651	ND
46.	82_O_58	Loknath Kandel	11/08/2082	Kathekhola 06, Baglung	-	ND
47.	82_O_59	Bala Nanda Kandel	11/08/2082	Kimbot 06, Kathekhola, Baglung	-	ND

Analyzed by:

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Bio-technologist Officer

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48.	82_O_60	Bhawani Sankhar Kandel	11/08/2082	Tapu 06, Kathekhola, Baglung	9845108835	ND
49.	82_O_65	Prem Prasad Koirala	24/08/2082	Lolunga 07, Bhirkot, Syangja	9841418954	ND
50.	82_O_66	Humanath Koirala	24/08/2082	Lolunga 07, Bhirkot, Syangja	9851021189	+D
51.	82_O_67	Fulmaya Koirala	24/08/2082	Lolunga 07, Bhirkot, Syangja	9846073281	ND
52.	82_O_68	Shyam Prasad Koirala	24/08/2082	Lolunga 07, Bhirkot, Syangja	9846205712	ND
53.	82_O_69	Bhuwani Koirala	24/08/2082	Lolunga 07, Bhirkot, Syangja	9846122070	+D
54.	82_O_70	Bikram Khawas	24/08/2082	Majhigaun 07, Bhirkot, Syangja	9805803743	+D
55.	82_O_71	Prem Prasad Koirala	24/08/2082	Lolunga 07, Bhirkot, Syangja	9841418954	ND
56.	82_O_76	Bishnu Devi Ranabhat	08/09/2082	Charangahira, Myagde 02, Tanahun, Tanahun	9846649570	ND
57.	82_O_77	Raj Kumar Shrestha	08/09/2082	Thansing Vanjyang, Myagde 02, Tanahun	9846062126	ND
58.	82_O_78	Bhoj Raj Sigdel (2 <sup>nd</sup> Contact: Nilkantha Sigdel)	08/09/2082	Jhagladhi, Shuklagandaki 11, Tanahun	9856060590	+D
59.	82_O_79	Nilkantha Sigdel, Naran Sigdel	08/09/2082	Jhagladhi, Shuklagandaki 11, Tanahun	9856060590	ND
60.	82_O_80	Nilkantha Sigdel	08/09/2082	Jhagladhi, Shuklagandaki 11, Tanahun	9856060590	ND
61.	82_O_81	Khem Raj Lamsal (2 <sup>nd</sup> Contact: Nilkantha Sigdel)	08/09/2082	Jhagladhi, Shuklagandaki 11, Tanahun	9856060590	+D
62.	82_O_82	Krishna Sharma Sapkota	08/09/2082	Lampata 07, Tanahun	9847645229	ND
63.	82_O_83	Jiwa Kumari Panta		Falewas 07, Parbat	9867627385	ND
64.	82_O_84	Sita Budathoki	24/09/2082	Mangala 05, Myagdi	-	ND
65.	82_O_85	Shyam Maya Budathoki	24/09/2082	Sikkum 05, Myagdi	9869216256	ND
66.	82_O_86	Ghadi Lal Sapkota	24/09/2082	Dhagel 05, Myagdi	-	+D
67.	82_O_87	Netra Prasad Adhikari	24/09/2082	Nuwagaun 05, Myagdi	9846001896	ND
68.	82_O_88	Lekh Bahadur Ale	08/10/2082	Harkapur Bikash Tol, Vyas 14, Tanahun	9814174982	ND
69.	82_O_89	Chandra Kumari Thapa	08/10/2082	Harkapur Bikash Tol, Vyas 14, Tanahun	9819157192	+D
70.	82_O_90	Netra Bahadur Thapa (Bato Mathi)	09/10/2082	Thin, Vyas 14, Tanahun	9804151918	ND
71.	82_O_91	Thaan Kumari Thapa (Bato Tala)	09/10/2082	Thin, Vyas 14, Tanahun	9806746636	ND
72.	82_O_92	Prem Bahadur Thapa	09/10/2082	Keshabtar, Thin, Vyas 14, Tanahun	9702719384	+D
73.	82_O_93	Khadka Bahadur Thapa (2 <sup>nd</sup> Contact: Prem Bahadur Thapa)	09/10/2082	Thin, Vyas 14, Tanahun	9702719384	ND
74.	82_O_94	Prem Bahadur Thapa	09/10/2082	Keshabtar, Thin, Vyas 14, Tanahun	9702719384	ND

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75.	82_O_95	Prem Bahadur Thapa	09/10/2082	Keshabtar, Thin, Vyas 14, Tanahun	9702719384	ND
76.	82_O_98	Bhakta Bahadur Paija	-	Baskharka, Jaljala, Parbat	9847641206	ND
77.	82_O_99	Bhakta Bahadur Paija	-	Baskharka, Jaljala, Parbat	9847641206	ND
78.	82_O_100	Jay Bahadur Sinjali	15/10/2082	Syalbas, Gallyang 05, Syangja	9847071843	ND
79.	82_O_101	Jay Bahadur Sinjali	15/10/2082	Syalbas, Gallyang 05, Syangja	9847071843	ND
80.	82_O_102	Bala Nanda Neupane	15/10/2082	Syalbas, Gallyang 05, Syangja	9849961745	ND
81.	82_O_103	Bala Nanda Neupane	15/10/2082	Syalbas, Gallyang 05, Syangja	9849961745	ND
82.	82_O_104	Ganga Bashyal	15/10/2082	Syalbas, Gallyang 05, Syangja	9856050030	+D
83.	82_O_105	Ganga Bashyal	15/10/2082	Syalbas, Gallyang 05, Syangja	9856050030	+D
84.	82_O_106	Tikaram Bashyal	15/10/2082	Syalbas, Gallyang 05, Syangja	9848111462	+D
85.	82_O_107	Tikaram Bashyal	15/10/2082	Syalbas, Gallyang 05, Syangja	9848111462	ND
86.	82_O_108	Som Nath Sharma	-	Bhimad 01, Chambot	9846701239	ND
87.	82_O_109	Som Nath Sharma	-	Bhimad 01, Chambot	9846701239	ND
88.	82_O_111	Aananda Dev Shrestha	-	Bhimad 05, Chisapaani	-	+D
89.	82_O_112	Dhiman Singh Thapa	-	Bhimad 02, ChyatDanda	-	+D
90.	82_O_113	Damodar Sigdel	-	Bhimad 01, Unsingh	-	+D
91.	82_O_114	Binod Sigdel	-	Bhimad 01, Chambot	-	ND
92.	82_O_115	Tol Bahadur Thapa	-	Bhimad 02, Salbaas	-	ND
93.	82_O_116	Til Bahadur Nepali, Gita Maya Nepali	-	Bhimad 08, Deurali	-	ND
94.	82_O_117	Kamal Bahadur Pokharel	-	Bhimad 09, Aaspadi	-	ND
95.	82_O_118	Chitra Kumar Gharti	-	Bhimad 08, Deurali	-	ND
96.	82_O_119	Shanta Nepali	-	Bhimad 08, Deurali	-	+D
97.	82_O_120	Ambar Bahadur Thapa	-	Bhimad 09, Aaspadi	-	ND
98.	82_O_121	Mit Bahadur Sharki	-	Bhimad 08, Deurali	-	ND
99.	82_O_122	Gir Bahadur Gharti	-	Bhimad 08, Deurali	-	ND
100.	82_O_123	Top Naath Jaisi	-	Bhimad 09, Aaspadi	-	+D
101.	82_O_124	Kesh Bahadur Chhetri	-	Bhimad 03, Aaspadi	-	+D

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102.	82_O_125	Khim Bahadur Thapa	-	Bhimad 09, Aaspadi	-	ND
103.	82_O_126	Jip Kumari Chhetri	-	Bhimad 09, Aaspadi	-	+D
104.	82_O_127	Purna Bahadur Thapa	-	Bhimad 09, Aaspadi	-	+D
105.	82_O_128	Muktinath Sharma Dhakal	-	Bhimad 08, Deurall	-	+D
106.	82_O_129	Rup Bahadur Sharki	-	Bhimad 08, Deurali	-	ND
107.	82_O_130	Gopal Shrestha	-	Bhimad 08, Deurali	-	ND
108.	82_O_131	Deurama Thapa	-	Bhimad 09, Jidi Bhanjyang	-	+D
109.	82_O_132	Nila Bahadur B.K.	-	Bhimad 03, Jidi Bhanjyang	-	ND
110.	82_O_133	Bishnu Bahadur Thapa	-	Bhimad 09, Jidi Bhanjyang	-	ND
111.	82_O_134	Bhoj Bahadur Thapa	-	Bhimad 09, Jidi Bhanjyang	-	ND
112.	82_O_135	Bom Bahadur Thapa	-	Bhimad 09, Jidi Bhanjyang	-	ND
113.	82_O_136	Balaram Gautam	-	Modi RM 07, Raamja	-	ND
114.	82_O_137	Naarayan Prasad Gautam	-	Modi RM 07, Raamja	-	ND
115.	82_O_138	Gopal Prasad Gautam	-	Modi RM 07, Raamja	-	ND

*Among 115 samples collected in total, we were able to detect HLB in 46 samples and not in 69 samples.*

**Remarks:**

The results generated are based on PCR analysis of randomly selected 3 to 4 leaves from each sample tree, collected at the provided date. DNA extracts when quantitatively analyzed had ~1.8 (260/280) absorbance ratio referring to high quality and purity of DNA. The samples relevant were collected from same tree, which strengthens the repeatability & uniformity of the diagnosis performed.

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