Screening of lentil (*Lens culinaris ssp. culinaris*) **germplasm for resistance to Stemphylium blight disease using qualitative characters**

ABSTRACT

In terms of both acreage and productivity, lentil is India's second most important pulse crop. Stemphylium blight, caused by *Stemphylium botrysum* Wallr., is a serious economic problem in eastern parts of India including West Bengal. As most of the cultivated lentil are susceptible to Stemphylium blight disease, the main alternatives for the control of the disease is to use highly toxic fungicides. The objective of this work is to evaluate the responses of 210 numbers of genotype collected from International Centre for Agricultural Research in the Dry Areas (ICARDA) in order to support the alternative disease management strategy. The qualitative attributes like days to flowering, height of the plant and leaf area were taken into consideration which were converted to qualitative attributes by using cluster analysis.. Along with that branching pattern and canopy types was also recorded as qualitative parameters. The result clearly indicated that days to flowering, leaf area, branching habit and canopy temperature significantly influence the disease development.

Keywords: Lentil, Qualitative traits, Stemphylium blight disease, Resistance

INTRODUCTION

India is the world's greatest producer and customer of pulses accounting 27 % of the whole production and about 30 percent of the whole consumption in the world. Lentil (*Lens culinaris ssp. culinaris* Medik.) is the world's fifth biggest pulse crop cultivated in additional 70 nations around the world, mostly in West Asia, North Africa, the Indian subcontinent, North America, and Australia. In India, lentil ranks second only to chickpea as one of the most nutritious cool-season eating legumes. During 2018-2019, it was grown on 1.51 million hectares in India, with an annual production of 1.56 million tonnes and a productivity of roughly 1032 kg/ha (Directorate of Economics and Statistics, 2018-2019).

Diseases are a major constraint to lentil production all over the world (Bayaa *et al.,* 1995). Among the diseases, Stemphylium blight (caused by *Stemphylium botrysum* Wallr) is a major one. The disease causes the loss of green part of leaf and reduction in photosynthetic capacity at flowering and pod filling stage affecting the quantity and quality of grain as well as seed production (Hay *et al.,* 2019). In India, 93% crop losses were recorded due to the disease (*Mandal et al., 2019*). The disease has been on the rise in frequency and intensity in India, which ranks first in lentil coverage areas globally. Under diverse environmental conditions, *S. botryosum* can infect a wide range of plant species. Temperature and moisture are the most critical environmental factors impacting *S. botryosum* conidial germination and disease progression on other hosts. It is a seed-borne disease that appears as small, light beige to brown lesions on leaves and leaflets above and below the canopy, often with angular light and dark brown areas, and causes severe leaf drop, resulting in defoliated vegetation and serving as a source of spores for future infection. Sometimes massive defoliation and stem bending was observed in severe cases (Alam *et al.,* 2017). Infected seeds are often stained and can have low germination rates. Many fungicides have been found to effectively control the fungal blight disease, with variable cost-benefit ratios (Das, 2015). Cultivating resistant cultivars is the best and most cost-effective approach to protect the crop from Stemphylium blight. The introduction of susceptible new cultivars could result in severe outbreaks of the

disease. As a result, the aim of the research is to screen lentil germplasm and evaluate the association between phenological and morphological features and disease resistance.

MATERIALS AND METHODS

The experiment was conducted during the year 2019-2020 at UBKV, Pundibari, Coochbehar, West Bengal, India. During the 2019-2020 growing season, a set of lentil germplasm with 210 genotypes collected from International Centre for Agricultural Research in dry Areas (ICARDA) were grown to see if disease severity is influenced by particular qualitative features. The phenotyping of the germplasm namely, days to flowering, plant height, compound leaf area, branch angle, and canopy types were considered. The seeds were sown in different blocks of 4 rows of 2m length of each genotype. Initially nitrogen (N), phosphorus (P_2O_5), potash (K_2O) fertilizers were applied at a rate of 20:40:0 kg ha-1. Irrigation was provided as required.

The following observations were recorded:

i) Days to 50% flowering: The number of days from the date of sowing required in which flowers in 50% of the plants first opened.

ii) Plant height: The plant height is the shortest distance between the upper boundary of the main photosynthetic tissues on a plant and the ground level. The plant was measured with a ruler starting from zero on the bottom.

iii) Branching angle: The branching angle is the angle between the main stem to the branches.

iv) Canopy: open canopy, closed canopy and wide canopy.

v) Leaf area: Leaf area is measured by using millimetre graph paper method.

vi) Grain yield: After harvesting from each plot, plants of each genotype is properly dried, threshed and cleaned properly. The grains were collected after threshing. Threshing is done bymachine.

vii) Disease scoring: The disease severity was indexed on a 0-10 disease scoring scale (Hashemi *et al.*, 2005) where, 0=Healthy plant; free of disease, 1=Dull leaves or few tiny tan spots, 2=A few small to large chlorotic spots, 3=Expanding lesions on leaves and leaf drop starting, 4=20% nodes on main stem showing chlorotic/necrotic symptoms and/or leaf drop, 5=40% nodes on main stem showing chlorotic/necrotic symptoms and/or leaf drop, 6=60% nodes on main stemshowing chlorotic/necrotic symptoms and/or leaf drop, 7=80% nodes on main and lateral stems showing chlorotic/necrotic symptoms and leaf drop, 8= 100% leaves dried up/defoliated but small green tip recovering, 9=100% leaves dried up/defoliated including tip but stem still green, and 10=Whole plant die and completely dried up.

viii) Percent Disease Index: The percent disease incidence (PDI) was calculated using the formula,

PDI = [Sum of numerical rating/total number of observations taken x maximum disease score] x 100Space issue

ix) Area under Disease Progress Curve (AUDPC): Area under Disease Progress Curve (AUDPC), measure the amount of disease as well as the rate of progress, was calculated by using formula (Das *et al.*, 1992). The value of AUDPC was

estimated using the midpoint rate or so-called trapezoidal integration method. The AUDPC has no unit.

$$AUDPC = \sum_{i-1}^{n-1} [(Xi + Xi + 1)/2] (ti + 1 - ti)]$$

Where, xi is the spot blotch severity on ith date, the ti is the ith day and n is the number of scoring days.

RESULTS AND DISCUSSION

In order to identify the resistant sources against the disease, the average Area Under Disease Progress Curve (AUDPC) in the clusters and the quantitative parameters accordingto the AUDPC cluster were arranged (Table-1). It indicates that the resistant plants were early in flowering, short and having large leaf area. Based on the data classification an attempt was taken to analyse the data using qualitative data analysis technique named as contingency analysis with Pearson chi- square test and estimating the level of significance of the classification based on frequency distribution.

The germplasm was categorized based on the said traits separately and contingency analysis was done using Pearson chi-square test to test dependency. The said parameters were categorized based on Ward's clustering method and accordingly cluster names were given to convert the quantitative data into qualitative data(fig.1). The disease was categorized in 5 classes based on AUDPC (<50, 50-150, 150-250, 250-350 and > 350) and were termed as Resistant, Moderately Resistant, Moderately Susceptible, Susceptible and Highly susceptible respectively. Similarly, days to flowering indicating the earliness of the genotype was also categorized as very early (<52 days), early (52-65 days), medium (65-85 days), late (>85 days). Plant height was also categorized as short (<24 cm), medium (24-27 cm), tall (>27). Branching pattern was categorized as erect or droopy, whereas canopy structure was divided into closed canopy and open canopy. Leaf area of the compound leaf was also classified into five clusters following the same method, which were small (<21 mm^2), medium (21-33 mm^2) large (>33 mm^2).

The Figure 2 depicts the frequency distribution of AUDPC through contingency analysis of different parameters with respective chi-square test and disease development in lentil is significantly dependent on days to flowering, compound leaf area and pattern of branching of the genotype. The early sowing generally escape infection as recorded in Nepal (Ghatri *et al.*,2014) and also wider spacing is recommended for reduced disease incidence (Darai *et al.*, 2017). The leaf area has been found higher in comparatively resistant genotypes as found in other crops like pear (Karklina *et al.*, 2021) however leaf area indexes have been found to be linearly correlated with yield under different pathosystems as sunflower (Liete *et al.*, 2006). Canopy architecture and density effects the spread andseverity of leaf disease as in rice (Zong *et al*., 2006), however, this character depends on the different parameters as branching pattern, nitrogen nutrition. Leaf erectness have been found to be positively related with resistance in wheat (Joshi *et.al.*, 2002) and in tea (Ponmurugan *et al*., 2019). The close canopy may influence the disease development by altering the microclimate of the plants including temperature, humidity, light transmission and thus makes it difficult to relate them with resistance (Zong *et al*., 2006; Srinivasachary *et al*., 2011).

CONCLUSION

To find the qualitative attributes of the crop related to its resistance to stemphyllum blight, a set of 210 genotypes were grown in the University farm with varying duration, plant type, and crop architecture. Quantitative attributes as days to flowering, height of the plant and leaf area were converted to qualitative attributes by using cluster analysis using Ward method. Along with that branching pattern and canopy types was also recorded as qualitative parameters. Contingency analysis with Pearson chi-square test was performed on the frequency tables and dependence of disease (AUDPC) on the said qualitative attributes were made. The result clearly indicated that days to flowering, leaf area and branching habit do influence the disease build up significantly at probability less than 5% and canopy structure influences at 5% level of significance. Thus, if we use these parameters to select genotypes from a huge population for resistance we can go with these qualitative attributes.

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Table-1: Average AUDPC of the clusters and days to flowering, plant height and leaf area of 206 genotypes grouped in 5 clusters.

Cluster	Number of genotypes	AUDPC	Days to Flowering	Plant Height	Leaf Area
1	13	30.00 ± 21.84	58.23 ± 14.61	24.25 ± 4.63	42.21 ± 12.03
2	41	121.42 ± 20.97	84.68 ± 17.49	26.72 ± 3.11	24.98 ± 13.40
3	71	192.09 ± 20.95	82.79 ± 16.33	26.22 ± 3.48	22.96 ± 8.27
4	69	281.10 ± 34.27	84.88 ± 17.82	26.04 ± 4.27	23.97 ± 8.54
5	12	408.61 ± 27.06	65.67 ± 21.02	28.63 ± 2.56	23.50 ± 9.82

Fig-1: Hierarchical Clustering of days to flowering, plant height and leaf area by Ward's method.

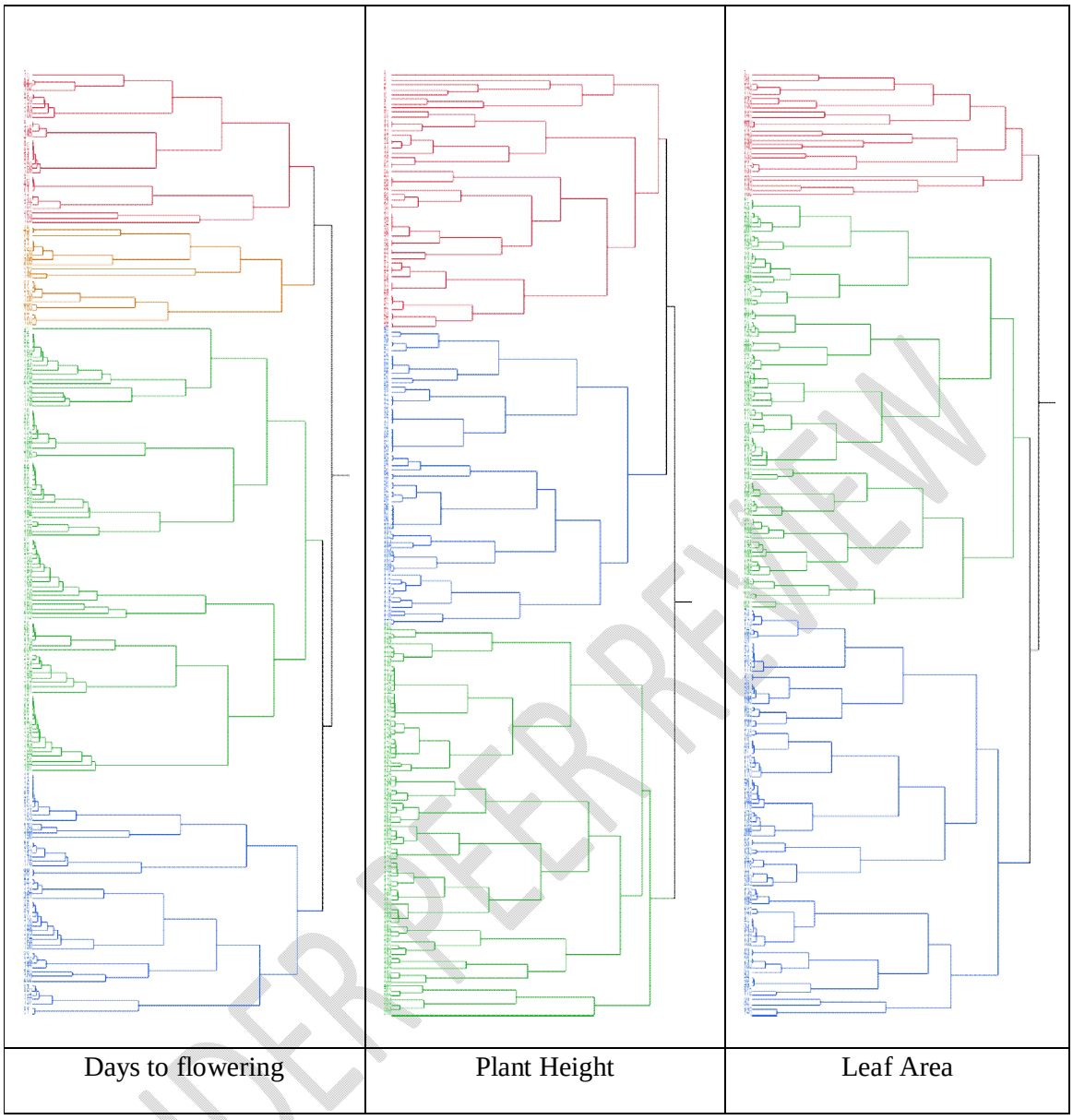
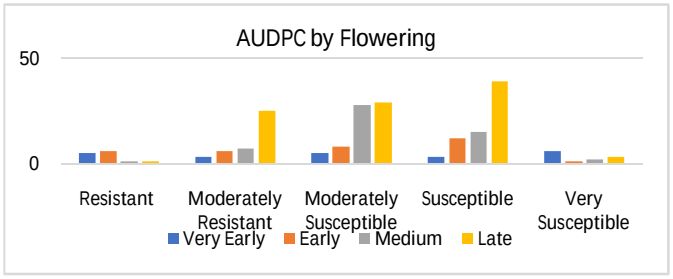
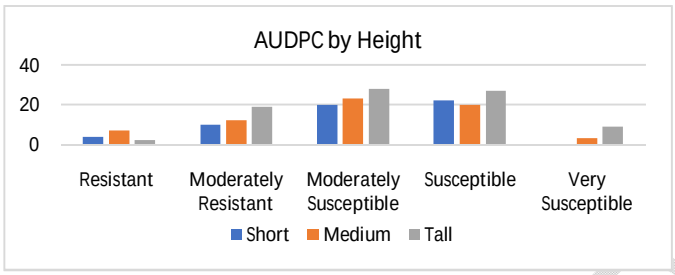
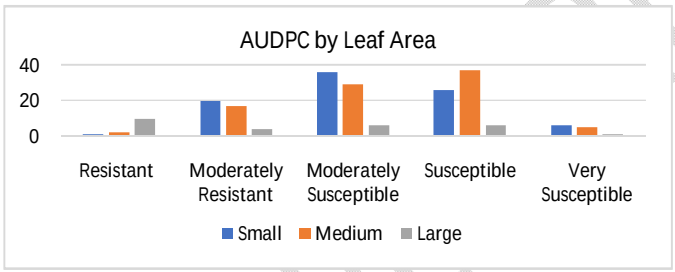
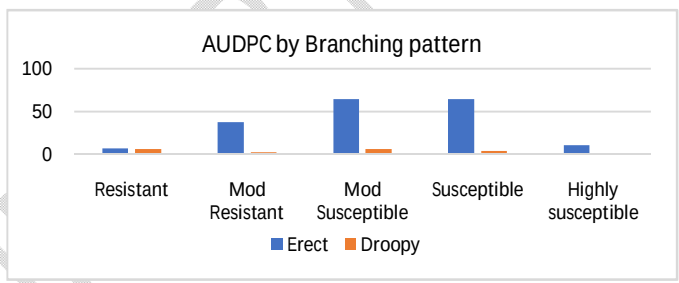
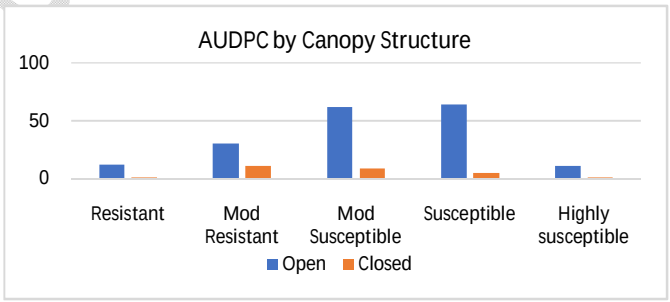


Fig-2: Frequency distribution of AUDPC through contingency analysis of different parameters with respective chi-square test.

	Pearson Chi square : 57.367 Probability Chi square <0.0001*
	Pearson Chi square : 12.27 Probability Chi square : 0.1393
	Pearson Chi square : 59.913 Probability Chi square <0.0001*
	Pearson Chi square : 21.324 Probability Chi square <0.0003*
	Pearson Chi square : 9.446 Probability Chi square : 0.0509

Supplementary Table

List of lentil genotype with pedigree

ENTRY_NO	Pedigree	ENTRY_NO	Pedigree
1	6037/10733/4-2	106	6994/10141/8-2
2	6002/LIRL-21-50-1-1-1/38-7	107	590/8461/2-4
3	6002/ILWL 118/1-1	108	4380/4372/7-4
4	6002/LIRL-21-50-1-1-1/38-5	109	8114/10956/22-5
5	6037/10733/4-4	110	5588/4372/4-5
6	6002/LIRL-21-50-1-1-1/14-1	111	8114/10956/12-2
7	6002/LIRL-21-50-1-1-1/14-5	112	813/4605/1-1
8	6002/LIRL-21-50-1-1-1/9-8	113	7716/7663/3-1
9	6037/10733/3-3	114	6994/10141/1-5
10	6002/LIRL-21-50-1-1-1/14-4	115	10016/10068/11-1
11	6002/LIRL-21-50-1-1-1/11-6	116	F1X2011S-132/F1X2011S-110/23-10
12	6002/LIRL-21-50-1-1-1/38-9	117	6002/7716/20-1
13	6002/LIRL-21-50-1-1-1/30-4	118	4380/4372/8-1
14	6002/LIRL-21-50-1-1-1/38-2	119	F1X2011S-132/F1X2011S-110/6-2
15	6002/99/209/2-5	120	813/4605/1-2
16	8008/LIRL-21-50-1-1-1/20-9	121	8008/10012/2
17	6002/7716/4-1	122	F1X2011S-

ENTRY_NO	Pedigree	ENTRY_NO	Pedigree
			132/F1X2011S-110/23-2
18	6002/LIRL-21-50-1-1-1/9-7	123	6994/10141/1-10
19	6002/7716/14-2	124	1005/10847/5
20	2313/4605/3-2	125	8114/10956/12-7
21	4605/ILWL 118/1SPS	126	8114/10956/16-9
22	4605/ILWL 118/2SPS	127	7010/09S 96565-1/1-2
23	4605/ILWL 118/3SPS	128	10866/10174/11SPS
24	4605/ILWL 118/6SPS	129	10867/10174/5SPS
25	4605/ILWL 118/9SPS	130	10867/10174/6SPS
26	4605/6002/5SPS	131	10012/590/4SPS
27	4605/6002/6SPS	132	10012/590/6SPS
28	4605/6002/7SPS	133	8114/7663/2SPS
29	4605/10848/5SPS	134	8114/7663/8SPS
30	6002/6994/11SPS	135	6002/6994/9SPS
31	6002/99/209/14SPS	136	10848/DPL 62/8SPS
32	1462/4372/11SPS	137	52/8461/2SPS
33	7986/ILWL074/10SPS	138	52/8461/3SPS
34	ILL4605	139	ILL10947
35	ILL4400	140	6002/LIRL-21-50-1-1-1/24-6
36	4380/4377/1-1	141	6002/LIRL-21-50-1-1-1/17-9
37	10675/8461/3-3	142	6002/LIRL-21-50-1-1-

ENTRY_NO	Pedigree	ENTRY_NO	Pedigree
			1/24-5
38	5883/8461/1-2	143	7716/7663/2SPS
39	10675/8461/4-4	144	358/10870/12-7
40	10675/8461/3-5	145	7531/8461/2-1
41	590/8461/1-4	146	7531/8461/1-5
42	6002/LIRL-21-50-1-1-1/3-9	147	7531/8461/3-4
43	590/8461/2-6	148	8114/7663/7
44	5588/4372/2-1	149	358/10870/6-2
45	6206/8461/8-2	150	10300/10061/5-1
46	10848/8143/1-2	151	4380/4372/3-6
47	8114/10956/11-5	152	10848/DPL 62/4-3
48	4380/4372/4-1	153	4605/8006/1-5
49	6206/8461/8-1	154	8114/7663/10-8
50	8008/10012/10	155	10865/10174/2SPS
51	4380/4372/16-1	156	10866/10174/4SPS
52	10848/LIRL-21-50-1-1-1/3-3	157	10866/10174/15SPS
53	8114/10956/14-8	158	10848/8114/5SPS
54	8114/10956/14-1	159	10012/2585/13SPS
55	8114/10956/9-8	160	6797/6816/2SPS
56	99/209/DPL 62/13-2	161	10072/1712/3SPS
57	8114/10956/22-9	162	6212/09S 96565-1SPS
58	8114/10956/9-2	163	6037/8006/5SPS
59	4380/4372/5-6	164	4605/7978/1SPS

ENTRY_NO	Pedigree	ENTRY_NO	Pedigree
60	DPL 62/8461/5-3	165	6994/DPL 62/4SPS
61	1875/8461/2-1	166	7978/DPL 62/7SPS
62	10140/DPL 62/6-1	167	7978/DPL 62/8SPS
63	8114/10956/4-5	168	LIRL-21-50-1-1-1/DPL 62/1SPS
64	10848/6994/1-2	169	LIRL-21-50-1-1-1/DPL 62/3SPS
65	7210/8010/7	170	LIRL-21-50-1-1-1/DPL 62/5SPS
66	8114/10956/12-8	171	99/209/DPL 62/6SPS
67	5588/8461/4SPS	172	4605/4380/2SPS
68	DPL 62/8461/6SPS	173	ILL5582
69	ILL2580	174	ILL5883
70	ILL7978	175	6002/LIRL-21-50-1-1-1/17-2
71	4903/5888/1-6	176	6002/LIRL-21-50-1-1-1/24-8
72	8406/8006/4-3	177	7978/DPL 62/1-1
73	10140/6994/6-9	178	LIRL-21-50-1-1-1/DPL 62/12-3
74	7978/ILWL 118/1-1	179	590/8461/3-7
75	4380/8461/1-3	180	LIRL-21-50-1-1-1/6994/3-6
76	7978/ILWL 118/4-2	181	6002/7716/9-1

ENTRY_NO	Pedigree	ENTRY_NO	Pedigree
77	4380/4372/8-9	182	6002/7716/14-1
78	1005/10847/3-2	183	LIRL-21-50-1-1-1/DPL 62/13-3
79	8114/10956/11-3	184	7978/DPL 62/10-8
80	1875/8461/6-2	185	5597/6797/10-4
81	10870/10871/9-10	186	5597/6797/10-8
82	6002/7716/2-2	187	LIRL-21-50-1-1-1/DPL 62/14-1
83	6994/10141/4-3	188	8114/7716/3-4
84	6994/10141/1-2	189	8114/7716/3-5
85	8008/10012/4-1	190	8114/7716/1-4
86	1875/8461/9-2	191	8114/7716/1-7
87	6994/10141/1-6	192	5597/6797/8
88	10848/6994/1-4	193	LIRL-21-50-1-1-1/DPL 62/11-7
89	7978/ILWL 118/2-2	194	10016/10068/4-1
90	6002/7716/20-4	195	LIRL-21-50-1-1-1/DPL 62/1-4
91	10072/1712/4-1	196	10072/1712/10-3
92	F1X2011S-132/F1X2011S-110/25-1	197	8114/7663/10-9
93	F1X2011S-132/F1X2011S-110/1-4	198	6002/7716/7SPS
94	F1X2011S-132/F1X2011S-	199	4605/LIRL 21-50-1-1-

ENTRY_NO	Pedigree	ENTRY_NO	Pedigree
	110/25-5		1/2SPS
95	F1X2011S-132/F1X2011S-110/25-6	200	10848/DPL 62/5SPS
96	813/4605/1-6	201	10848/DPL 62/14SPS
97	6037/8006/6SPS	202	10848/DPL 62/16SPS
98	6002/LIRL-21-50-1-1-1/4SPS	203	7978/LIRL-21-50-1-1-1/9SPS
99	10848/99/209/1SPS	204	7978/99/209/1SPS
100	7978/LIRL-22-107/1SPS	205	10220/8461/5SPS
101	LIRL-21-50-1-1-1/DPL 62/8SPS	206	4605/2684/4SPS
102	2313/4372/1SPS	207	4605/3596/2SPS
103	7986/ILWL074/2SPS	208	ILL8006
104	ILL4605	209	ILL6821
105	ILL590	210	WBL 77