



EDA procedure: An Effective Tool in the Study of Inoculation Medium for *in vitro* Seed Germination in Eleven Different Varieties of *Carica papaya* L.

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ABSTRACT

In vitro seed germination was studied in eleven varieties of *Carica papaya* L. Seven different inoculation media were selected and time taken (in days) to germinate was recorded. The data so collected was analyzed by EDA procedure rather than the conventional inferential procedures. EDA procedures were found to give very quick and useful results with ease in computation. Median Polish recommended that MS and B5 were better choice for inoculation media.

KEY WORDS: *Carica papaya* L., *in vitro* seed germination, inoculation media Exploratory data analysis (EDA), Median Polish (MP), Box - Whiskers plot, Two-way Analysis of variance (ANOVA)

INTRODUCTION

Carica papaya L. is a commercially important plant, being cultivated widely for the consumption of fresh fruits and for use in drinks, jams, candies and as dried crystallized fruits [1]. *Carica papaya* L. belongs to the family Caricaceae. Papaya seeds are rather short lived. The viability of seeds depends on suitable storage. Various genotypes behave differently regarding seed germination and seedling vigour and the quality of papaya seeds deteriorates very fast if left to dry with its sarcotesta intact [2].

In the present study an attempt was made to study the effect of different media on time taken (in days) for *in vitro* seed germination in different varieties of *Carica papaya* L. and select the best inoculation media. Eleven varieties of *Carica papaya* L. (CO-2, CO-5, CO-6, Coorg Honey Dew, Disco, Madhubindu, Madhur, Pusa dwarf, Ranchi, Red Lady and Washington) and seven different inoculation media ($\frac{1}{2}$ MS, LS, W, WPM, MS+B5, B5 and MS) were selected.

As these data were in batches rather than some representative population, EDA procedures were applied which has been developed by [3]. There are number of EDA procedures [4], like graphical visualizations, Median Polish and Box Whiskers plot [5], these procedures have changed the language and paradigm of Statistics. They are flexible inductive and look at the data in as many angles as possible in outlook for some interesting features. Set of stringent conditions, validity of underlying assumptions and concern about outlying observations are the main hurdles in confirmatory data analysis (CDA). Where as EDA procedures i) are not unduly affected by outlying observations, ii) do not insist for the mandatory tests prior to the analysis and iii) in addition they work as complementary tools for statistical inference of CDA.

MATERIALS AND METHODS

Seeds of eleven varieties of *Carica papaya* L. (CO-2, CO-5, CO-6, Coorg Honey Dew, Disco, Madhubindu, Madhur, Pusa dwarf, Ranchi, Red Lady and Washington) were selected. Seeds were soaked in water for 24 hours at room temperature and then their seed coats were removed. After the removal of seed coat, the seeds were inoculated on different inoculation media (MS, $\frac{1}{2}$ MS, B5, WPM, W, LS or MS+B5). Data was collected for *in vitro* seed germination (in days).

For statistical analysis the data so collected was analyzed by EDA procedure rather than the conventional inferential

procedures. EDA procedures were found to give very quick and useful results with ease in computation. For the present study graphical visualizations, Median Polish and Box Whiskers plot were mainly used. Statistical software used was Minitab.

RESULTS AND DISCUSSION

Seeds (after the removal of seed coat) of all the eleven varieties of *Carica papaya* L. germinated between 23 to 42.3 days, 101.3 to 134.5 days, 41.3 to 46 days, 84.6 to 99.3 days, 80.1 to 88 days, 73.2 to 82 and 90 to 95.6 days when inoculated on MS, 1/2 MS, B5, LS, W, WPM and MS+B5 media respectively (Table 1).

Table 1: Effect of different inoculation medium on germination (in days) of seeds in different varieties of *Carica papaya* L.

Varieties	Time taken for germination (d)						
	Inoculation medium						
	MS	1/2 MS	B5	LS	W	WPM	MS+B5
Co-2	32.0±0.46	130.7±0.17	42.3±0.49	95.3±0.20	88.0±0.32	77.3±0.41	91.6±0.27
Co-5	27.6±0.44	101.8±0.19	41.3±0.49	90.4±0.29	82.7±0.37	73.2±0.44	90.6±0.29
Co-6	36.3±0.48	101.3±0.08	43.6±0.49	96.3±0.18	86.3±0.34	77.3±0.41	91.3±0.28
CHD	37.3±0.48	124.4±0.30	45.0±0.49	96.0±0.19	87.3±0.33	80.3±0.39	94.0±0.23
D	35.3±0.47	127.7±0.34	46.0±0.49	96.0±0.19	86.0±0.34	74.7±0.43	94.3±0.23
MB	30.6±0.46	101.3±0.08	43.6±0.49	97.6±0.15	82.7±0.37	74.1±0.43	95.6±0.20
MA	29.3±0.45	124.4±0.30	41.3±0.49	96.0±0.19	81.1±0.39	74.1±0.43	93.3±0.25
PD	37.6±0.48	126.3±0.15	42.3±0.49	94.3±0.23	82.3±0.38	74.6±0.43	93.0±0.25
RA	40.6±0.49	127.0±0.15	45.0±0.49	98.5±0.12	87.3±0.33	82.0±0.38	91.6±0.27
RL	42.3±0.49	134.5±0.24	46.0±0.49	99.3±0.08	88.0±0.32	79.6±0.40	92.6±0.26
W	23.0±0.42	103.3±0.23	42.3±0.49	84.6±0.36	80.1±0.39	73.2±0.44	90.0±0.30

Values are mean of three sets of determinants. Each set with 100 seeds.

Table 2: Tukey's Test for pair of inoculation media.

Medium	1/2MS - 1	LS - 2	W - 3	WPM - 4	MS+B5 - 5	B5 - 6	MS - 7
Mean	118.427	94.936	84.682	76.400	92.536	43.518	33.809
Diff from 1		23.491	33.745	42.027	25.891	74.936	84.618
2			10.254	18.536	2.4 NS	51.418	61.127
3				8.282	-7.854	41.164	50.873
4					-16.136	32.882	42.591
5						49.018	58.727
							9.709

Comparison of Two-way ANOVA and Median Polish

y_{ij} = i^{th} observation from j^{th} column, was used to denote time taken to germinate for the i^{th} variety when applied j^{th} treatment (inoculation medium). For both the methods similar additive model is used. Response, y_{ij} = General effect + effect due to i^{th} Treatment + effect due to i^{th} variety + e_{ij} (error component) $i=1, 2, \dots, 11$ and $j=1, 2, \dots, 7$.

$\hat{y}_{ij (Two-way)}$ was fit due to Two-way ANOVA

$\hat{y}_{ij (MP)}$ was fit due to Median Polish

$y_{ij} - \hat{y}_{ij (Two-way)} = r_{ij (Two-way)}$ was residual due to Two-way ANOVA and

$y_{ij} - \hat{y}_{ij (MP)} = r_{ij (MP)}$ was residual due to Median Polish.

1. The box plot $y_{ij}, \hat{y}_{ij (Two-way)}, \hat{y}_{ij (MP)}$ was drawn for each treatment (Fig 1).
2. The residuals $r_{ij (MP)}$ was plotted against the

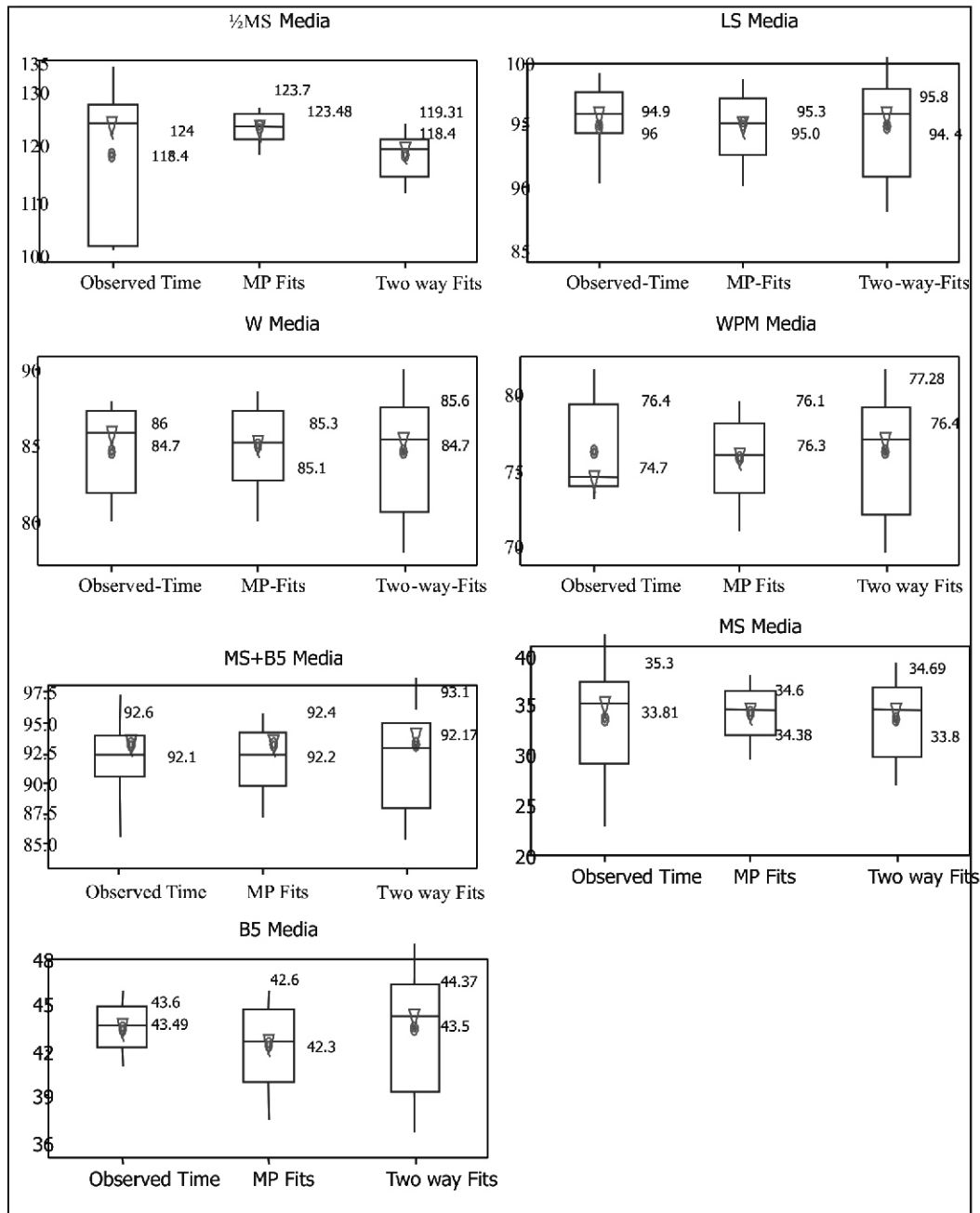
- i) Treatment (inoculation media) effect (Fig 2)
- ii) Variety effect (Fig 3)

Results from Box-Plot

It was seen that MP fits were more appropriate to the given data

- In most of the cases it was symmetric, with less spread.
- Values on plot indicated that 1/2MS medium had a very high waiting time followed by MS+B5, LS, W, WPM. B5 and MS were at the least rank.

Fig 1: Comparison between Median Polish and Two way Model



- All varieties showed less scattered points, hence all varieties showed uniform response to inoculation medium.
- MP Residuals were very near to zero
- MP estimates were almost unbiased estimates of time to germinate or slightly over estimate of waiting time but very rarely underestimate.

Results from graph of residuals showing effect of varieties in different inoculation media

- MP Residuals showed no serious erratic behavior except 1/2MS medium. It could be taken as an indication of homogeneity of variances of treatments.
- The validity of model adequacy was also verified by EDA. Where as in Two-way analysis it was necessary to carry out the test of non-additivity [6] for no interaction between variety and media.

Pair wise comparison of inoculation media

Tukey had suggested EDA and CDA should run in harness with one complementing other. Tukey's test [7] was applied based on studentised range statistic for testing difference between treatment means (Table 2). The Tukey's

Fig 2: MP residuals for eleven varieties of *Carica papaya* L. (CO-2, CO-5, CO-6, Coorg Honey Dew, Disco, Madhubindu, Madhur, Pusa dwarf, Ranchi, Red Lady and Washington)

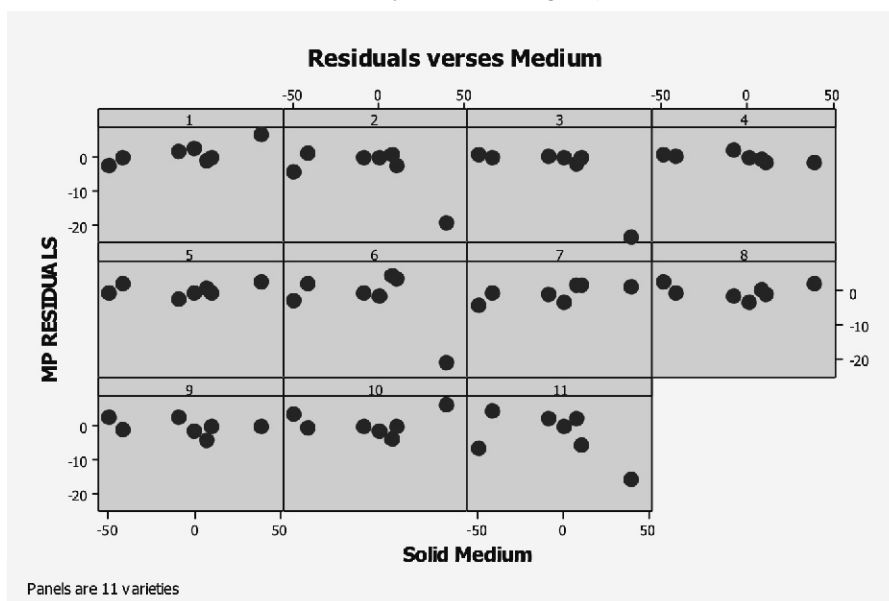
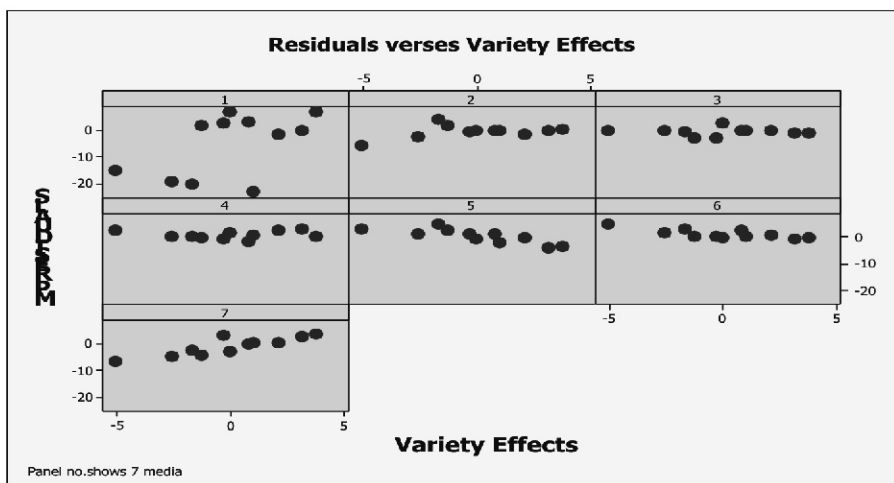


Fig 3: MP residuals for seven different inoculation media (1/2MS, LS, W, WPM, MS+B5, B5 and MS)



test indicated no difference between means of $\frac{1}{2}$ MS and MS+B5. All other pairs differed significantly.

CONCLUSION

As discussed above for such a layout of experiments, EDA gave quite satisfactory solutions. Here Median Polish was not only as competent as ANOVA, but was better than it. Present study showed that $\frac{1}{2}$ MS should never be recommended as inoculation medium. Rank MS+B5 and LS, W, WPM as inoculation media according to time taken (days) to germinate. Thus based on EDA procedures MS and B5 media were recommended as better choice for inoculation media for the *in vitro* seed germination in eleven different varieties of *Carica papaya* L.

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