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Are plants with anti-cancer activity resistant to crown gall?: a test of hypothesis

Bioprospecting for plants with anticancer activity has been a major focus in the search for plant-based cures[1]. The National Cancer Institute (NCI) alone has reportedly screened over 35,000 plant species for anti-cancer activity[2–6]. Two of the three most important anti-cancer compounds available today, namely taxol and camptothecin, were the result of this endeavour[7–9]. The screening of a large number of plants over dozens of cancer cell lines has often been time-consuming[5].

In an effort to minimize the screening process and hasten the pace of drug discovery, the NCI developed a number of rapid bench-top bioassays to short-list potential plants, which then could be targeted for more advanced screening[10]. One of these bench-top bioassays was the crown gall tumour assay (CGTA)[11]. Crown gall is a neoplastic syndrome caused by *Agrobacterium tumefaciens*. Crown gall is a common disease of dicot plants, including many woody shrubs and various herbaceous plants[12]. Infected plants exhibit tumorogenic growth symptoms in stem collars and other parts of the plant. The bioassay, based on this disease evaluates the ability of plant extracts to inhibit tumours induced by *A. tumefaciens* in model systems such as potato tuber discs[13]. The rationale for employing this bioassay rests on the fact that the tumorogenic mechanism induced by *A. tumefaciens* in plants is in many ways similar to that of animals[14,15]. McLaughlin and Rogers[16] have shown an association between the inhibition of crown gall formation on potato discs and the *in vivo* 3PS anti-tumour activity by the plant extracts. Accordingly, the use of this bioassay has helped in the short listing of plants with anti-cancer activity, and in the discovery of novel compounds from plants[10,13,17–19].

Thus it appears that plants intrinsically resistant to crown gall infection could, in principle at least, also be associated with anti-cancer activity. A test of prediction and demonstration of an association between crown gall resistance and anti-cancer activity could have immense exploratory potential in the search for newer plants as sources of anti-cancer activity.

Towards this end, we compiled a database of 1193 species (comprised of 588 genera and 138 families) of dicot plants based on their resistance or susceptibility to crown gall infection as reported by DeCleene and DeLey[20]. Species were assigned a qualitative score of either crown gall resistant (+) or susceptible (–), as reported by DeCleene and DeLey[20]. For the purpose of this analysis, we used data on only those 1110 species for which the information was complete.

We then compiled a list of 38 plant species that were reported to possess anti-cancer activity from a variety of published sources (Plants for future Database, www.pfaf.org, etc.; supplementary information available upon request from the authors). All studies sourced here were based on either an *in vitro* or an *in vivo* assay for anti-cancer activity.

Based on these two datasets, we performed a contingency chi-square test of association between anti-cancer activity and crown gall resistance. Ideally, such a test would require the setting up of a 2×2 matrix of crown gall (resistance/susceptibility) and anti-cancer activity (present/absent) and then statistically evaluating the association. Unfortunately, because of a well-recognized positive bias in publications[21,22], papers often only report studies where anti-cancer activity was observed, seldom publishing studies with no activity. Consequently, a 2×2 matrix with data cells corresponding to crown gall resistance/susceptibility and anti-cancer activity (absence) is deficient, thus limiting the association analysis.

Under these circumstances, we analysed the association by generating the expected frequency distributions for crown gall resistance or susceptibility using the data from DeCleene and DeLey[20]. For each of the 38 plants, we inferred their resistance or otherwise, to crown gall from the database assembled from DeCleene and DeLey[20]. We analysed the association at both the species and genera level. At both levels, there was significant association of crown gall resistance and anti-cancer activity (chi-square (at species level) = 10.63, $P < 0.001$; chi-square (at genera level) = 12.70, $P < 0.001$; Table 1). Further, as against 81% of the species resistant to crown gall from those that had anti-cancer activity, only 41% of the species was resistant from the random collection represented by DeCleene and DeLey[20].

Thus, our results indicate that plants with anti-cancer activity appear to have a higher proportion of species resistant to crown gall than randomly selected species. Though not as directly demonstrative as would have been desired through a 2×2 association analysis, our results nevertheless provide a useful first step in working towards a more robust test of the association.

Crown gall tumour assay has been in vogue as a rapid technique for screening for anti-cancer activity in plants. However, there has not been any explicit test of an association between crown gall resistance and anti-cancer activity. Our results pave the way for the development of algorithms that make the search for anti-cancer activity in plants in a more directed manner. These results seem to support the view of Smith[23], expressed nearly 92 years ago when he stated, 'It is my belief that a diligent study of tumours

Table 1. Association between resistance or susceptibility of plants to crown gall disease and their anti-cancer activity. Data are provided at both the species and genera level

	Species		Genus	
	Observed frequency	Expected frequency	Observed frequency	Expected frequency
Resistant to crown gall	31	21	31	20
Susceptible to crown gall	7	17	7	18

Chi-square (at species level) = 10.63, $P < 0.001$; Chi-square (at genera level) = 12.70, $P < 0.001$.
Note: Expected frequencies for the cells have been generated based on data given in DeCleene and DeLey[20] on random collection of plants with resistance or susceptibility to crown gall.

in plants will help to solve the cancer problem'.

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Does recent migration explain elevated blood pressure? A study among migrants in Delhi, India

Hypertension has emerged as a major public health problem in developing countries. The association between urbanization and blood pressure is well-known. However, studies dealing with immediate impact of migration are limited in developing countries[1–3]. Here, we have tried to explore the differences between recent-migrants and settled-migrants with regard to hypertension.

The settled-migrants (who have settled and are residing in Delhi since at least 10 years) were sampled from a resettlement colony in South Delhi (Dakshinpuri extension, Dr Ambedkar Nagar) while the recent-migrants (who had migrated to the city of Delhi from rural villages within the last two years, this being their first migration) were selected from slums (Prabhu Basti, Indira Camp, Khirki Gaun) and work sites (construction work sites at Chirag Dilli and South District Office complex construction work site at Saket). Sample size was estimated according to Lwanga and Lemeshow[4]. With a confidence level of 95% and an absolute precision of ten percentage points on either side of the true value of the difference between the proportions, the estimated sample size was 193 in each group[4]. The sample size was rounded-off to 200 in each group. Five blocks of the resettlement colony were selected randomly for the sample of settled-migrants. In each block, four streets from four directions were selected. In each street, two random points were chosen, and from each random point five individuals (both men and women) were selected randomly in order to attain a minimum sample of 200 individuals. The eligibility criteria for participation were that the subject should belong to the migrant group and residing in Delhi since a minimum of 10 years; he/she should be aged 20 years or more. The recent-migrants were selected from three slums and in addition, due to non-availability of eligible respondents during the daytime, we visited two construction work sites where they work. This approach has been adopted to attain a minimum sample of 200 individuals. In each slum, community leaders and members were contacted to identify the newly migrated individuals. The identified individuals who were aged 20 years and above were contacted and after confirming that they had migrated within two years from rural villages and that it was their first migration, they were consi-