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

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Abstract

The Crown gall tumour assay (CGTA) is one of several bench top bioassays recommended for the rapid screening of plants with anti-cancer activity. The rationale for the use of the bioassay is that the tumorogenic mechanism initiated in plant tissues by *Agrobacterium tumefaciens* is in many ways similar to that of animals. Several plant species with anti-cancer activity have already been discovered using this bioassay. However till date no explicit test of an association between anti-cancer activity of plants and their resistance to crown gall formation has been demonstrated. Demonstration of an association could have exploratory potential when searching for plants with anti-cancer activity. In this paper, we determined whether or not a statistically significant association between crown gall resistance and anti-cancer activity exists in plants found in existing published data sets. Our results indicate that plants with anti-cancer activity have a higher proportion of their species resistant to crown gall formation compared to a random selection of plants. We discuss the implications of our results especially when prospecting for newer sources of anti-cancer activity in plants.

Key words: anti-cancer activity, crown gall resistance, NCI

Introduction

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], [3], [4], [5], [6]. Two of the three most important anti-cancer compounds available today, namely taxol and camptothecin, were the result of this endeavor [7], [8],[9], [10].The screening of a large number of plants, involving over half-a-dozen solvent extraction systems, and testing them on 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 assays to short-list potential plants, which then could be targeted for more advanced screening [11]. One of those bench top bioassays was the crown gall tumour assay (CGTA) [12]. Crown gall is a neoplastic plant disease caused by *Agrobacterium tumefaciens* [13]. Infected plants, exhibit tumorgenic growth symptoms in stem collars and other parts of the plant. Crown gall is a common disease of dicot plants including many woody shrubs and various herbaceous plants. In this bioassay, the ability of plant extracts to inhibit tumours induced by *A tumefaciens* in model systems such as potato tuber discs is evaluated [14]. 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 [15], [16]. The use of this bioassay has resulted in many short lists of plants with anti-cancer activity, and has helped with the discovery of novel compounds from plants [17], [18], [14], [11], [19]. McLaughlin et al. (1991) indeed were able to show an association between the inhibition of crown gall formation on potato discs and the *in vivo* 3PS anti-tumour activity by the plant extracts.

From the above results, it follows that plants intrinsically resistant to crown gall infection could, in principle at least also be associated with anti-cancer activity. To the best of our knowledge, there have been no attempts to evaluate this hypothesis. A test of the 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. We have examined the association between crown gall resistance and anti-cancer activity in plants and now report on the results of that study.

Materials and Methods

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 in Cleene et al., (1976). Species were assigned a qualitative score of either crown gall resistance (+) or susceptible (-). 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 and other references mentioned in Table 1). 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 analyzed the average proportion of species resistant to crown gall: Using a bootstrap analysis involving repeated sampling with replacement (PopTools version 2.6.2); [20], we randomly selected 100 species from the database and determined the proportion of species resistant to crown gall. The process was repeated 100 times. For each of the repeats, we computed the proportion of species resistant to crown gall. A frequency distribution of the proportion of species resistant to crown gall was then plotted and the overall mean proportion of species resistant along with the standard deviation was computed. The proportion of species resistant to crown gall from among the 38 species reported to possess anti-cancer activity was calculated. For each of the 38 plants, we inferred their resistance or otherwise to crown gall from the database assembled from Cleene et al., (1976) and computed the proportion of species that were resistant.

Finally, we performed a test of significance between the two proportions (for species drawn randomly *vs* species possessing anti-cancer activity) using a one-tailed student t-test.

Results and discussion

The frequency distribution of proportion of species resistant to crown gall for the randomly drawn species (N=100 from 1110 species, repeated 100 times) was nearly normally distributed, with an overall mean proportion of 0.41 ± 0.051 (Figure 1). The proportion of species resistant to crown gall among plants exhibiting anti-cancer activity (N=38) was 0.81, which was significantly higher than that of randomly selected plants (one tailed t-test, $p<0.001$).

Plants with anti-cancer activity therefore appear to have a higher proportion of species resistant to crown gall than randomly selected species. While the result suggests that an association between crown gall resistance and anti-cancer activity in plants exists, a more robust demonstration would have been to set up a 2 X 2 matrix of crown gall (resistance/susceptibility) and anti-cancer activity (present/absent) and then statistically evaluating the association. Demonstration of such an association may have more accurately shown if plants with anti-cancer activities are more likely to be found in plants that are resistant to crown gall compared with a randomly chosen set of species. Unfortunately, because of a well-recognized positive bias in publications, papers often only report studies where anti-cancer activity was observed, seldompublishing studies with no activity. Consequently, a 2 X 2 matrix with data cells corresponding to crown gall resistance/susceptibility and anti-cancer activity (absence) is deficient thus limiting the association analysis.

Though not as directly demonstrative as would have been desired, our results nevertheless provide a useful first step in working towards a more robust test of the association. Results of further analysis could pave the way for the development of algorithms that make the search for anti-cancer activity in plants in a more directed manner.

Acknowledgements

Work was partially supported by grants from the Department of Biotechnology, Govt of India. Prof N. V. Joshi, Centre for Ecological Sciences, Indian Institute of Science, Bangalore offered critical suggestions on the analysis. Prof K. Veluthambi, Madurai Kamaraj University, Madurai drew our attention to the crown gall data in Cleene et al.,(1976).

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Figure and table legends

Figure 1: Frequency distribution of proportion of species resistant to crown gall formation in randomly selected plants (see text for details). The mean proportion of species resistant to crown gall for a random collection of plants and that for plants with anti-cancer activity is also indicated.

Table 1: List of plant species with anti-cancer activity along with information on crown gall resistance or susceptibility.

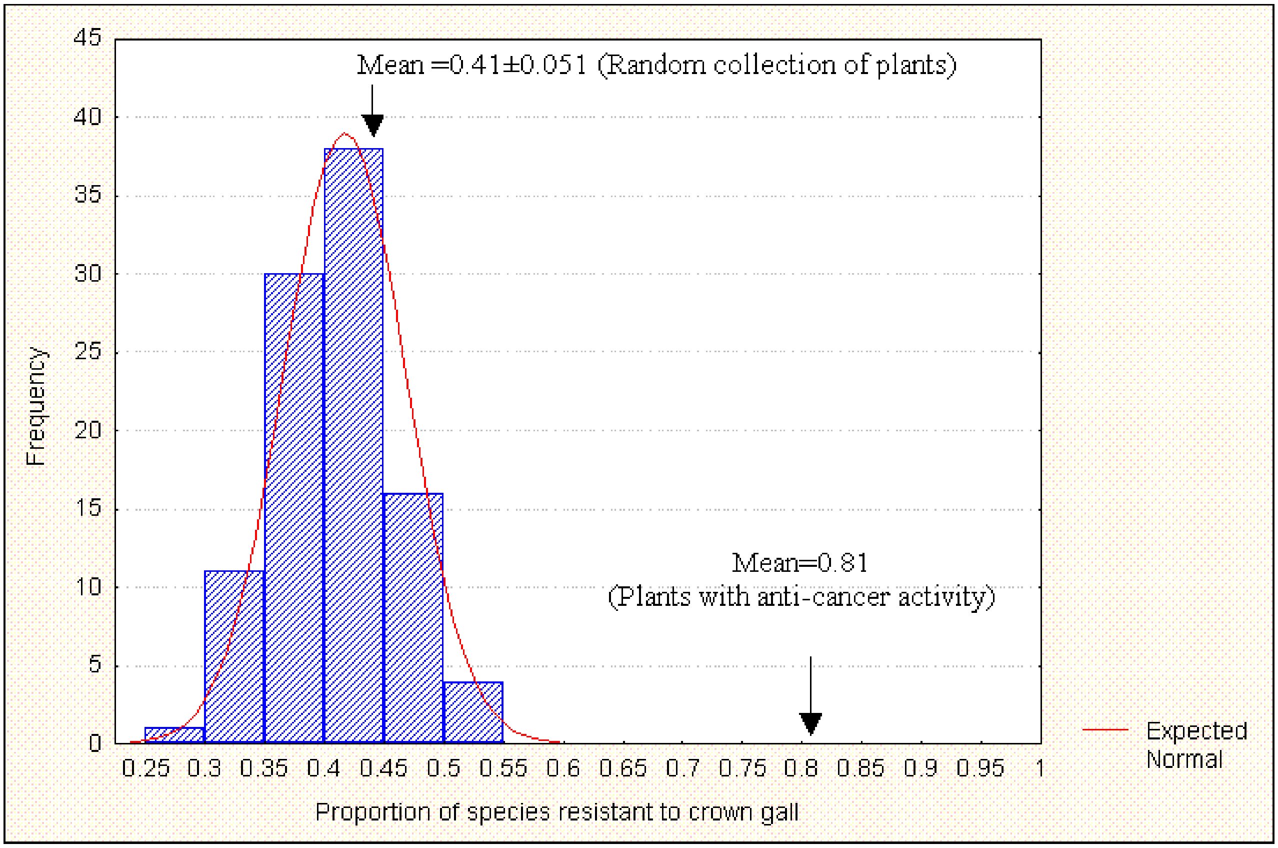

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Table 1: List of plant species with anti-cancer activity along with information on crown gall resistance or susceptibility.

Sl no	Common name	Scientific name	Family	Anti-cancer activity		Crown gall resistance or suceptiblity (inferred from Cleene et al., 1976)
				Reference	**In vivo or in vitro anticancer assay**	
1	Aster	*Aster sp*	Compositae	[21]	Epifriedelinol shows anti-cancer activity	Resistant
2	Birch	*Betula alleghaniensis*	Betulaceae	[22]	Induces apoptosis in human melanoma and neuroblastoma cells	Resistant
3	Blueberry	*Vaccinium sps*	Ericaceae	[23]	Induces Phase-II Xenobiotic detoxification enzymes	Resistant
4	Cactus	*Opuntia microdasys*	Cactaceae	[24]	Induces apoptosis and cell cycle arrest of cancer cells.	Resistant
5	Dahlia	*Dahlia rosea*	Compositae	[25]		Resistant
6	Gossypium	*Gossypium*	Malvaceae	[26]	Cytotoxic to murine B16 melanoma and L1210 lymphona cells	Resistant
7	Hydrangea	*Hydrangea serrata*	Hydrangeaceae	[25]		Resistant
8	Maple	*Acer sp*	Sapindaceae	[27]	Possesses activity against Walker 256 and Sarcoma 180 cell lines	Resistant
9	Rhododendron	*Rhododendron indicum*	Ericaceae	[28]	Cytotoxic against Spodoptera frugiperda cell line Sf-9	Resistant
10	Sequoia	*Sequoia sempervirens*	Taxodiaceae	[29]	Shows Brine Shrimp Lethality	Resistant
11	Spruce	*Picea sps*	Pinaceae	[30]	Inhibits growth of LNCaP tumors in Mice.	Resistant
12	Maidenhair tree	*Ginkgo biloba*	Ginkgoaceae	[31]	Inhibits DNA damage	Resistant
13	Golden-rain tree	*Koelreuteria paniculata*	Sapindaceae	[32]	Tyrosine kinase inhibition	Resistant
14	Holly	*Ilex aquifolium*	Aquifoliaceae	[33]	Ursolic Acid Inhibits Cyclooxygenase-2 Transcription in Human Mammary Epithelial Cells	Resistant
15	Hornbeam	*Carpinus betulus*	Betulaceae	[34]	Active against human melanoma cells	Resistant
16	Serviceberry	*Amelanchier spp.*	Rosaceae	[35]	Berry extract induces cell-cycle arrest.	Resistant
17	Barberry	*Berberis vulgaris*	Berberidaceae	[36]	Berberine affects the structure of filamentous actin cytoskeleton of the B16 cells.	Resistant

18	Mahonia	*Mahonia fremontii*	Berberidaceae	[37]	Protoberberine shows antimutagenic activity by inhibiting Topoisomerase I	Resistant
19	Linden	*Viburnum dilatatum*	Caprifoliaceae	[38]	Iridoids glucosides exhibits moderate inhibitory activity against HeLa S3 cancer cells	Resistant
20	Larch	*Larix decidua*	Pinaceae	[39]	LaPSvS1 showed good antiangiogenic activity in CAM-assay.	Resistant
21	Magnolia		Magnoliaceae	[28]	Induces apoptosis in lukemia cells	Resistant
22	Pine	*Pinus sp*	Pinaceae	[7]	Cell cycle arrest: inhibits tubulin diassembly	Resistant
23	Douglas fir	*Pseudotsuga menziesii*	Pinaceae	[40]	Induces growth inhibition in human lung carcinoma cells	Resistant
24	Bald Cypress	*Taxodium distichum.*	Cupressaceae	[41]	Cell cycle arrest: inhibits tubulin diassembly	Resistant
25	Hemlock	*Conium maculatum*	Umbelliferae	[42]	Inhibits malignant tumours especially breast cancer.	Resistant
26	Redbud	*Cercis canadensis*	Redbud	[42], [25]	Antilukemia	Resistant
27	Smoke tree	*Cotinus coggygria*	Anacardiaceae	[43]	Gallic acid has been shown to display selective cytotoxicity against tumor cells, and to induce apoptosis in tumor cells.	Resistant
28	Yew	*Taxus baccata*	Taxaceae	[7]	Cell cycle arrest: inhibits tubulin diassembly	Resistant
29	Andromeda	Andromeda sp	Ericaceae	[25]		Resistant
30	Mimosa	*Albizia julibrissin*	Leguminosae	[24]	Shows marked inhibitory action against Bel-7402 Cancer cell line.	Resistant
31	Mountain laurel	*Kalmia latifolia*	Ericaceae	[44]	Shows cytotoxicity against 9KB cell lines	Resistant
32	Euonymus	*Euonymus alatus apterus*	Celastraceae	[21]	Dulcitol inhibits growth of cancerous cells	Suceptible
33	Rose	*Rosa roxburghii*	Rosaceae	[45]		Suceptible
34	Russian- olive	*Elaeagnus angustifolia*	Elaeagnaceae	[46]	Inhibits several stages in colon carcinogenesis.	Suceptible
35	Almond	*Prunus dulcis*	Rosaceae	[47]	Betulinic acid showed antiproliferative activity toward MCF-7 cells (GI_{50} = 0.27μM)	Suceptible
36	Walnut	*Juglans sps*	Juglandaceae		Plumbagin is a potent inhibitor of the NF-B activation.	Suceptible
37	Ficus	*Ficus citrifolia*	Moraceae	[48]		Suceptible
38	Wisteria	*Wisteria sinensis*	Fabaceae	[25]		Suceptible